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THE UNIVERSITY OF ALBERTA

AN ELECTROPHYSIOLOGICAL STUDY OF AFFERENT
VAGUS NERVE PATHWAYS

A DISSERTATION

SUBMITTED TO THE FACULTY OF MEDICINE
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF BACHELOR OF SCIENCE (MEDICINE)

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

by

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ABSTRACT

Interest in the reflex control of blood volume has led, in this investigation, to the attempted electrophysiological study of the distribution of afferent vagal nerve fibers in the rootlets of the vagus nerve, in the tractus solitarius, and on the cells of the nucleus solitarius.

Small bipolar silver pickup electrodes under oil, and glass microelectrodes in a saline environment were used in the studies, on 20 cats, of the peripheral nerve. Both methods proved to be unsatisfactory. Since this investigation, a study by Bonvallet and Sigg has been read; this describes the distribution of cardiac and respiratory neurones in the vagal rootlets.

Extracellular central nervous system recording in the region of the tractus and nucleus solitarius was accomplished in 22 cats using glass micropipettes, of tip diameter 2-20 μ and resistance 0.5 -5.0 megohms, coupled to a suitable high impedance amplifying system. A cathode ray oscilloscope display of the nervous activity was photographed on high speed recording paper. All electrode positions were checked histologically. The patterns of nervous activity recorded were classified as: respiratory, "cardiovascular" types, continuous, random, and artefactual. The conclusion was reached that none of these recordings represented the activity of primary afferent neurones of the vagus but that all recorded central activity originated in the somae of central

Introduction

It has been suggested that left atrial vagal receptors are the nerve endings initiating a reflex which controls blood volume (25, 26, 30, 31, 32, 65, 66). Valuable information concerning the central connections of the afferent vagal fibres involved in this reflex would be obtained if the atrial fibres could be selectively ablated or stimulated at some point above the nodose ganglion. This possibility led to an attempt to demonstrate a separation of the afferent vagal fibres into anatomical groups representing separate functional modalities. The problem was approached by using electrophysiological techniques.

It was thought that the vagal rootlets and the tractus solitarius of the medulla would be likely places to search for such a separation into functional groups. Should such an arrangement not prove to be the case, it was still believed that the investigation of the distribution of function within the more proximal primary vagal tract would be of intrinsic value.

Some of the published literature that was thought relevant to the investigation of the distribution of the afferent visceral information to the brain stem was reviewed. This included reports of the location of the receptors, their function, and pattern of discharge. The known anatomical structure of the vagal nerve trunk and the tractus and nucleus solitarius was reviewed because of the influence this was liable to exert on the techniques needed to study these structures electrophysiologically.

In view of the special interest attached to the thoracic vagal receptors a short review was made of the knowledge of the centres of respiration and vasomotor control in the brain stem. The thoracic vagal receptors of respiratory and cardiovascular function must be in physiological connection with the motor centres concerned with the regulation of these types of activity, hence, knowledge of these centres could aid in the investigation of the distribution of the afferent fibres.

The review revealed that knowledge of the distribution of the vagal afferent fibres within the brain stem is uncertain and knowledge of the distribution of fibres of differing function is scanty and limited to one group of experiments in which compound action potentials were recorded. Unit activity within the tractus and nucleus solitarius had not to our knowledge been reported in the literature at the commencement of this investigation.

A short review of the methodology of central nervous system investigation was prepared because current concepts of neural organization necessarily depend on the methods of study. It was hoped that this review would aid the interpretation of any records obtained from the central nervous system.

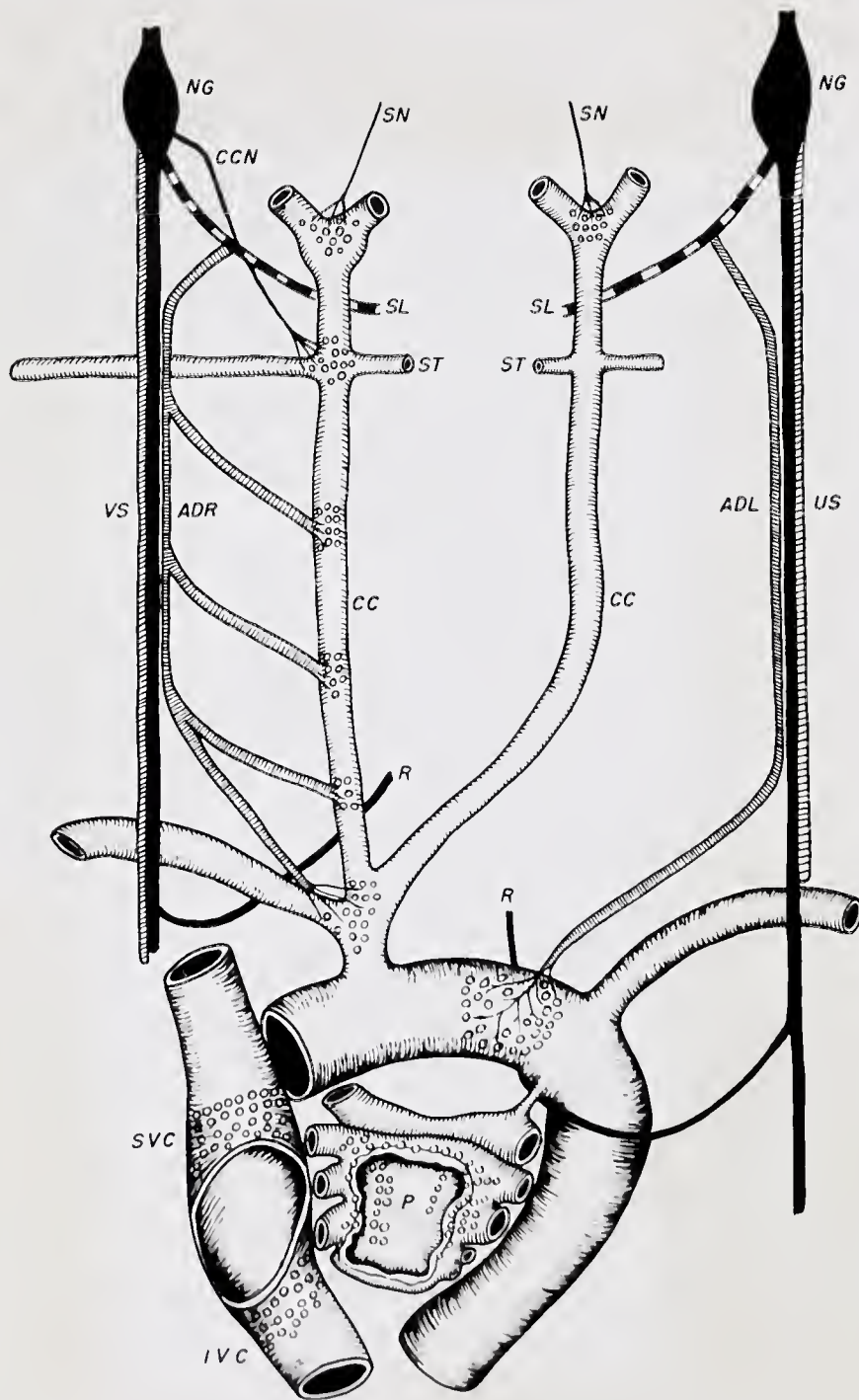


Fig. 1

Diagram of the thoracic receptor sites in the cat. ADL, left aortic depressor nerve; ADR, right aortic depressor nerve; CC, common carotid artery; CCN, common carotid nerve; IVC, inferior vena cava; NG, nodose ganglion; P, pulmonary arteries; R, recurrent laryngeal nerve; SL, superior laryngeal nerve; SN, carotid sinus nerve; ST, superior thyroid artery; SVC, superior vena cava; VS, vagosympathetic trunk. Open circles represent the receptor sites.

THE RECEPTORS OF THE SENSORY FIBRES OF THE VAGUS NERVE AND THE
REFLEXES THEY INITIATE

Receptors in the Thorax

The reflexes arising from receptors in blood vessels, the heart and the lungs have been classified into four groups by Aviado and Schmidt (6). It will be assumed that each of the separate reflexes represents a separate set of receptor endings and, hence, a separate set of fibers in the vagus nerve. Admittedly, differing reflex effects could be caused by stimulation of the same receptor by differing degrees of stimuli, but this proposition will not be considered because it represents an unnecessary complication of the presently known facts.

The classification has been modified slightly to include only those reflexes resulting from stimulation of vagal sensory receptors. The four types of reflexes are:

1. Receptors producing complete inhibitory effects.

Receptors in the Aortic Arch.

2. Receptors producing incomplete inhibitory effects.

- (2A) Receptors in the Cardiac Walls
- (2B) Receptors in the Pulmonary Veins
- (2C) Receptors in the Lung Parenchyma
- (2D) Receptors in the Pulmonary Artery and Cardiac Walls
- (2E) Receptors in the Common Carotids proximal to the sinuses.

3. Receptors producing complete excitatory effects.

Chemoreceptors of the Aortic Body.

THE HISTORY OF THE UNITED STATES OF AMERICA

BY JAMES M. SMITH

The history of the United States of America is a story of growth and development. It begins with the first settlers who came to the New World in search of a better life. They found a land of opportunity, but also a land of challenges. The early years were marked by struggle and hardship, but the spirit of the pioneers was strong. They built a nation from scratch, one that was based on the principles of liberty and justice for all. The story of the United States is a story of the triumph of the human spirit over adversity. It is a story of the courage and determination of a people who refused to be defeated. The United States has come a long way since its founding, but the values that guided its early years remain its strength. The story of the United States is a story of hope and possibility. It is a story that inspires and motivates people around the world.

THE HISTORY OF THE UNITED STATES OF AMERICA

BY JAMES M. SMITH

THE HISTORY OF THE UNITED STATES OF AMERICA

1776	July 4th	Declaration of Independence
1787	September 17th	Constitution signed
1791	September 16th	Bill of Rights adopted
1800	January 1st	Capital moved to Washington
1820	March 3rd	Missouri Compromise
1861	April 9th	Lincoln's Emancipation Proclamation
1865	April 9th	Lincoln's Second Inaugural Address
1876	November 3rd	Reconstruction Act
1890	September 9th	Wheeler-Howard Act
1914	June 15th	Antitrust Act
1917	April 6th	War Revenue Act
1918	October 3rd	War Relocation Act
1920	January 1st	Prohibition Act
1933	December 8th	Banking Act
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THE HISTORY OF THE UNITED STATES OF AMERICA

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4. Receptors producing incomplete excitatory effects.

(4A) Receptors in the lower respiratory passages
and lung parenchyma

(4B) Receptors in the great veins and right atrium.

This classification will now be applied to the thoracic
receptors.

1. RECEPTORS PRODUCING COMPLETE INHIBITORY EFFECTS

Receptors of the Aortic Arch

Position: In their review Aviado and Schmidt (6) state that the
receptor endings are found in the coronary orifices, the root of
the brachio cephalic trunk, and the aorta as far as the beginning
of its descending portion. Heymans and Neil (34) give essentially
the same information based on work by Tello on mice embryos
(Tello, J. F. (1924), Trab. Lab. Invest. Biol. Univ. Madr. 22:
295)). The left aortic nerve has its adult distribution through-
out the aortic arch with nerve endings which are filamentous
arborizations of the larger nerve fibers. The endings are situated
in the adventitia of the vessel (55). The right "aortic nerve"
arises from the subclavian carotid angle and the neighboring part
of the brachiocephalic artery. Studies by Muratari (6) on the
rabbit contain results similar to those of Tello.

Stimulus and discharge pattern

Pressure and distortion are probably adequate stimuli
for the aortic arch pressoreceptors, but this is not absolutely
certain (6). Whitteridge (81) has observed the discharge pattern

of these endings from single fiber preparations and he notes that when the peak frequency of discharge is plotted against the systolic pressure there is sometimes a departure from linearity due to concurrent changes in intrapleural pressure. Neil (21) has shown pulsatile stimulation to be more effective than steady stimulation at an equivalent mean pressure level in producing reflex effects. Hence the effect on receptor discharge of changes in pulse contour can also have significance. Sometimes a depressor receptor will fire with a superimposed respiratory rhythm. This has been related to an unusually large respiratory oscillation in blood pressure, with its peak at the end of expiration.

Reflex response

Heymans and Ladon in their double-dog experiments in 1924 & 1925 showed that a rise of blood pressure produces a reflex bradycardia (34). I. de Burgh Daly and Verney in 1926 showed that a rise of aortic pressure specifically caused bradycardia, while Kahn (34) passed a sound wave down the brachiocephalic artery causing distention at its junction and a reflex hypotension. Anrep and Starling (34) in 1925, using a convincing double-dog experiment, showed that a rise of pressure in the aorta caused a reflex dilatation in the cephalic vessels. Nakayama in 1953 and Neil in 1956 (34) have shown that a rise in pressure in the aortic arch will cause systemic hypotension, hypopnea and a bradycardia.

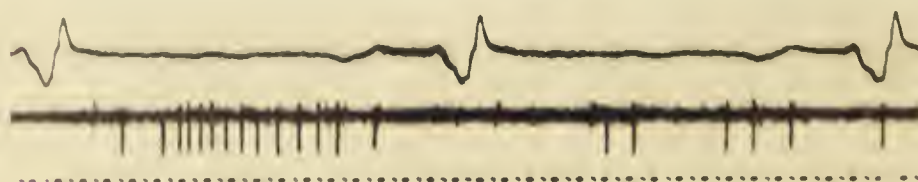
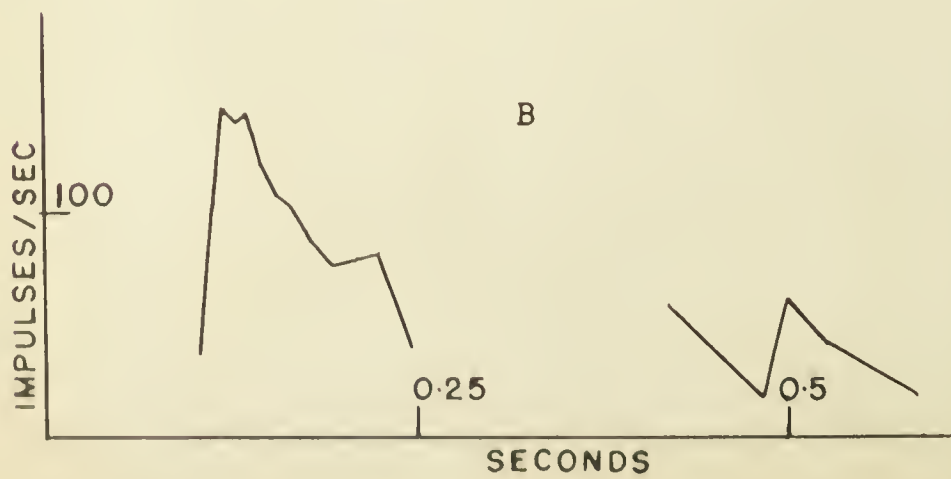
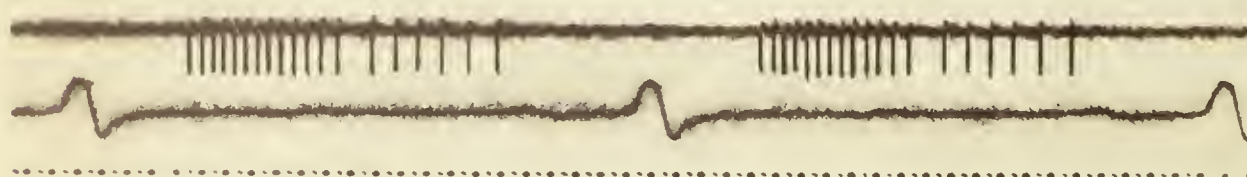
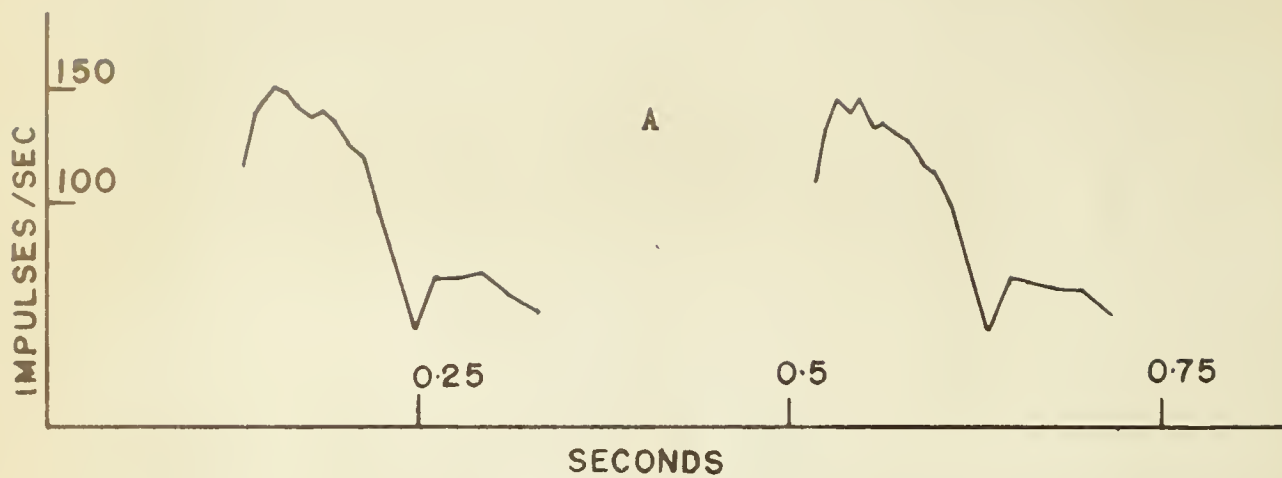


Fig. 2 (Records obtained from the dog)

Typical cardiovascular receptor responses recorded from the cervical vagus nerve.

A. From top to bottom: frequency plot of aortic depressor response, aortic depressor nerve response, ECG, time marker 120/sec.

Frequency of nerve discharge is plotted as the reciprocal of the time interval between two adjacent spikes. This value is plotted against time and is located at the time of occurrence of the second action spike. For convenience in plotting the frequency-time curves, the nomogram described by K. M. Chapman was used (WADC Technical note 57-371. USAF 1957).

B. From top to bottom: frequency plot of left atrial receptor response, ECG, atrial nerve response, time marker 120/sec.

Records loaned by Dr. J. W. Pearce.

2. RECEPTORS PRODUCING INCOMPLETE INHIBITORY EFFECTS

(2A) Receptors in the cardiac walls

Position: Sensory endings in the heart can be divided into four groups (6).

(a) Subendothelial endings which are entirely supplied by the vagus nerve except in the frog. The endings are encapsulated, flattened, or nodal depending on the staining technique used (1). The endings are grouped mainly in the atria at the entrance of the large veins and Nonidez (56) believes these to be responsible for reflexes arising from the right atrium.

(b) Myocardial endings which in the atria are perimuscular arborizations and in the ventricles are spindles somewhat similar to those found in skeletal muscle. Nonidez (56) describes these endings.

(c) Subepicardial endings which are brush-like and have unknown central connections.

(d) Coronary endings have been described (35) along the arteries and their branches in the myocardium. Their terminal divisions are unmyelinated filaments, some of which extend directly into the walls of the arteries. These endings are sympathetic in the cat but vagal and sympathetic in the dog, according to McEachern et al (7).

Pressoreceptors in the heart have been localized physiologically into at least two main regions (6).

(a) In the right side of the heart predominantly but not exclusively in the atrium.

(b) In the left side of the heart, predominantly but not exclusively in the ventricle.

Coleridge et al have described the location of the atrial receptors in the dog (14). Branched myelinated fibers 3-10 μ in diameter are described in the endocardium in the junctional regions between the venae cavae and the right atrium and between the pulmonary veins and the left atrium.

Stimulus and Discharge Pattern

The effective stimulus for most of the receptors in the cardiac walls seems to be an increased pressure inside the chambers (6). However, Paintal (59) and later, Henry and Pearce (32) described left atrial receptors in the dog which discharged with the increase of volume of the chambers rather than the pressure. It is conceivable, therefore, that the receptors are activated by the amount of physical distortion they undergo, by whatever means this distortion is produced.

Paintal has described the response pattern of the ventricular receptors (63), and says that these are characterized by an early systolic burst of impulses which terminate before mid systole. The fibers are of the A group of medullated fibers with a mean conduction velocity estimated at 10-20 meters per second. In this paper Paintal summarizes in table form the characteristics useful in identifying various cardiovascular afferent fibers.

The ventricular receptors also respond to drugs such as the veratrum alkaloids to give the Bezold Jarish reflex (63).

Paintal (59) describes two types of receptors in the left atrium of the cat. The receptors of type A fire with the "a" venous pressure wave while type B shows no "a" volley but has a late systolic pulse. However, Pearce, Henry and Chapman (66) have pointed out that receptors showing a "type B" activity can shift to the early systolic timing of Type A receptors after hemorrhage or on increased intrathoracic pressure.

Reflex Response

Avaído (6) has shown that receptors in the right side of the heart give rise to reflex hypotension and bradycardia with no respiratory effects being constantly shown. It is believed that the pressoreceptors in the left ventricle cooperate with those in the systemic arteries to maintain aortic blood pressure while the right atrial "pressoreceptors" are stabilizing the pulmonary arterial pressure or blood flow.

Gaver and Henry (25, 26) believe that the atrial receptors provide information from the low pressure system to the higher centers responsible for cardiovascular and body fluid homeostasis. This reflex effect is accomplished by increased diuresis as a response to increased atrial volume.

Hymans and Neil (34) have criticized a paper by Henry et al (31) in which other reflexes, contraction of the spleen and peripheral veins and the release of vasoconstrictor substances

following hemorrhage, are attributed to changes of activity in the atrial receptors. While little direct evidence exists in support of these additional reflex effects it has been shown by Chapman (13) in an argument based on the information theory of Claude Shannon that there is a superabundance of information provided by the atrial receptors for control of a function such as diuresis. Even allowing for a necessary redundancy, sufficient information seems to be available for reflex effects other than those concerned with diuresis.

Although sensory receptors exist in the coronary vessel walls (35), the position of receptors activated by any particular stimulus is in doubt owing to the possible response of myocardial receptors to ischaemia. The reflexes involved have been investigated with regard to myocardial infarction (6). Both sympathetic and parasympathetic pathways are believed to be involved, but the nature of, or even the presence of reflex action has not been established.

(2B) Receptors in the pulmonary veins

The Receptors

The receptors have been classified on the basis of their reflex activity (6).

1. The veins contain receptors producing reflex hypotension and those respiratory effects, namely apnea followed by polypnea, encountered during increased blood pressure in the lung vessels.

2. The pulmonary capillaries and venules contain receptors which are activated by emboli. However, the reflex arcs are believed to be local involving nerve cells located in the mediastinum.

That these endings are different from the Hering-Breuer stretch receptors is shown by their different reflex effects despite the observation (15) that a change occurs in stretch fibre activity on increasing the blood volume in the lungs.

Stimulus and response characteristics

The exciting stimulus is an increase in pressure in the pulmonary vessels. Embolization can also stimulate the reflex activity for a variety of possible reasons: the pulmonary arteriolar pressure may rise, mechanical irritation may cause stimulation or liberation of chemical substances at the site of embolization, and the blood pressure distal to the embolus may fall.

Reflexes

The reflex effects described above, hypotension with apnea followed by polypnea have not been evoked in experiments in which the central end of the vagus was stimulated (6). Varied effects on the circulatory and respiratory systems have been noted in this case. This, however, only reflects the diverse sensory components of the vagus nerve. The many components have sometimes confused the interpretation of electrical recordings of pressoreceptor activity (67). The records which Pearce and Whitteridge believed to represent activity in receptors in the lung vessels

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have since been shown to arise in the atria (34).

The presence of reflexes originating in the venous circulation of the lungs evidences the widespread distribution of baroreceptor endings through the cardiovascular system. These baroreceptor systems are similar to those arising in the carotid sinus and aortic arch but are less highly developed (6).

(2C) Lung Parenchyma

Position of endings

The location of the endings in the lungs has been divided into three areas depending on the associated reflex activity (6).

1. Cough reflex arising from excitation of the tracheobronchial passages. (These are included with receptors of type 4A).
 2. Hering-Breuer reflexes from stretch receptors in the lung parenchyma.
 3. Presso-reflexes from the pulmonary vessels.
- Elftman (23) has arranged the receptor endings in the lung parenchyma as follows:
1. Coarse deeply staining endings in the respiratory bronchiols.
 2. Flattened endings along the alveolar ducts.
 3. Paddle endings at the air sac bifurcation.
 4. Complex branched nodular terminations in the alveolar wall.

5. Delicate straight or coiled terminations in the alveolar wall.

These endings all have vagal connections.

The morphology of the endings has been described by Larsall (47).

Hering-Breuer reflexes

Hering and Breuer in 1868 described the respiratory effects of volume changes in the lungs. They found that inhibition of respiration on inflation of the lungs or respiratory stimulation on deflation of the lungs depended on the vagi being intact.

Stimulus and response characteristics

At least three functional types of such endings have been described (1, 6).

1. Slowly adapting endings stimulated by inflation. These receptors fire at a maximum natural frequency of from 50- 100 cycles/sec as recorded in single fibre preparations of the vagus nerve. These endings do not fire during expiration except under conditions of pulmonary congestion (1, 15).

2. Rapidly adapting endings stimulated by deflation and by overinflation.

3. High threshold receptors stimulated by extreme inflation only.

Reflex response

Head (29) has described the responses as:

1. Normal inflation inhibiting respiration.
2. Extreme inflation exciting inspiration.
3. Moderate deflation exciting inspiration.

(2D) Receptors in the Pulmonary artery conus and cardiac walls.

1. Pulmonary artery conus

Aviado et al (5) have described endings in the pulmonary artery conus. These endings are supplied by the vagus and by pulmonary branches of the sympathetic (6).

The effective stimulus to these endings may be an increase in pressure in the pulmonary conus (6), and a reflex response to such increase in pulmonary arterial pressure is a bradycardia if the vagi are intact, or a tachycardia if the vagi are cut. The latter response is mediated by the sympathetic system. No vasomotor or respiratory effects are included in these reflexes.

2. Cardiac walls

The position of these receptors is described in the earlier section on the cardiac walls, 2A. The endings in this case have both a vagal and a sympathetic nerve supply, but like those described earlier, are excited by distention of the heart chambers. Reflexly these endings innervated by the vagus give rise only to a bradycardia (6).

(2E) Receptors in the carotids proximal to the sinuses

Green has described (27, 28) a baroreceptor area in the common carotid artery which is supplied by the vagus nerve. The

receptor area in the common carotid artery lies between the level of the superior thyroid artery and the subclavian bifurcation. These areas are supplied by nerve fibres which join to form the aortic depressor nerve on the right side (34). Boss and Green in 1956 (10) studied these endings and found that they consisted of fibrils in the inner adventitia.

An effective stimulus to these endings is increased intracarotid pressure; the typical response is hypotension due to vasodilatation with the heart rate and respiration remaining unaffected (6).

3. Receptors producing complete excitatory effects

Chemoreceptors of the aortic body

Heymans has described (34) the work performed on the structure of the aortic body, the fibers of which travel in the aortic depressor nerve when this exists separately. Howe has described the blood supply to the aortic bodies and has recorded the nervous activity originating in the aortic bodies near the root of the left subclavian arteries (36, 37). It has been shown that these structures, like the bodies beneath the aortic arch, contain chemoreceptors.

The stimuli to which these bodies are sensitive are: acidosis, hypercapnia, hyperthermia, cyanides, sulfides, nicotinic drugs, and the veratrum alkaloids (6). The reflex response is hypertension, due to vasoconstriction, and tachycardia, hyperpnea and, sometimes, convulsions.

4. Receptors producing incomplete excitatory effects

(4A) Receptors in the lower respiratory passages and lung parenchyma

Lower respiratory passages

The reflexes involved with stimulation of receptors in this region were mentioned in the preceding section on the lung parenchyma, 2C. Pulmonary branches of the vagus supply these nerve endings which are stimulated by inhalation of irritant gases and vapours. Stimulation gives rise to reflex coughing or to an expiratory blast. No circulatory effects have been reported.

Lung parenchyma

These endings described under section 2C are rapidly adapting and are stimulated by extreme inflation or deflation and by embolization (6). An increased respiratory rate constitutes the reflex responses; no circulatory effects occur. Embolization causes pulmonary vasoconstriction, it is possible that this effect is a manifestation of sympathetic nervous activity.

(4B) Receptors in the great veins and the right atrium

Bainbridge (6) originally proposed the existence of receptors in the venae cavae and the right atrium, and such have since been found. The sporadic and inconsistent evidence of reflexes attributed to these endings has, however, made their identification difficult. The reflex responses claimed to result from stimulation of these endings include tachycardia (Bainbridge), vasoconstriction (McDowall), and hyperpnea (Harrison).

Aviado (6) is of the opinion that these reflexes are of

historical interest but are probably nonphysiological. An effective stimulus to the endings is an intravenous injection of blood, plasma, or saline. If pressoreceptors are involved then the increased intravascular pressure is the stimulus, if chemoreceptors are involved then gas content, acidity, ionic balance, or viscosity may be the responsible factors.

The possible role of receptors in the regulation of blood volume has already been referred to, although it is not yet clear whether these endings belong here or in the earlier group of Aviado and Schmidt stated to be responsible for incomplete inhibitory effects.

Receptors in the abdomen

Several workers have shown the important role of the vagus nerve in carrying information on the distention of the gastrointestinal tract and other abdominal viscera (40, 41, 54, 61, 79).

Iggo in 1955 performed experiments on the goat and the cat and noticed two different receptors in the stomach.

1. A group that produced a sustained discharge on distension, and also had a resting discharge.
2. A group have an intermittant discharge, both resting and not resting.

Iggo concludes that these receptors are tension recorders in series with muscle fibers.

Paintal (64) has described the responses from the mucosal

mechanoreceptors in the small intestine of the cat. The mechanoreceptors are innervated by vagal fibers in the small intestine and show a spontaneous activity of periodic trains of pulses.

Hubbard has produced a study of rapid mechanical events in a mechanoreceptor (38). The receptor was a Pacinian corpuscle obtained from the mesentery. His conclusion that the rapid adaptation of the corpuscle is a direct consequence of its mechanical properties may apply also to mechanoreceptors of other types, as for example in the lungs.

Chemoreceptors in the gastrointestinal tract have been described by Iggo and Paintal. Iggo (42, 43) has described endings which are stimulated by acid (0.1N HCl) or alkaline solutions (0.1N NaOH). The thresholds were pH 3 for the acid type of ending and pH 8 for the basic type. These endings were not responsive to hypotonic or hypertonic solutions nor to other chemicals. The endings adapt slowly and are not mechanoreceptors. It follows that these endings must differ from the stretch endings that Paintal (62) found to respond to drugs such as phenyl diguanide, 5-hydroxytryptamine, nicotine, adrenaline, and glucose.

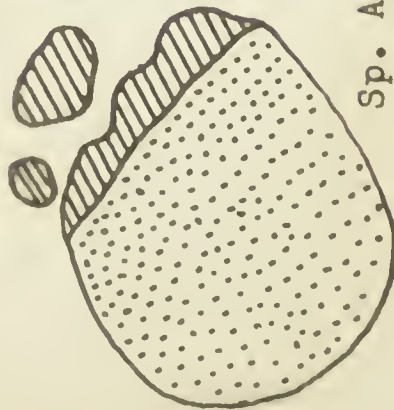
Other receptors from abdominal viscera may be implied to exist by the coordinated evacuation of the gall bladder, the varying volume of the spleen and the evidence of contribution to vascular regulation by the abdominal vasculature. How much of this information is carried in sympathetic pathways is at present unclear.

Dorsal Rootlets



Ventral Rootlets

B. Acc.



Sp. Acc.

Fig. 3

Distribution of motor and sensory components in the vagal rootlets.

Black, motor; white, sensory; lined, bulbar accessory (B. Acc.); dotted, spinal accessory (Sp. Acc.).

After Foley and DuBois, J. Comp. Neurol. 60: 137, 1934.

The Vagus nerve

The vagus nerve of the cat like that of man possesses two ganglia, the jugular and the nodose. The smaller jugular ganglion is situated in the jugular foramen. From it arises the auricular branch, a somatic sensory nerve with its cell bodies located in the jugular ganglion.

The nodose ganglion is a spindle shaped enlargement of the vagus trunk distal to the jugular ganglion. From its middle or lower part arises the superior laryngeal nerve, which, except for its external ramus, is a sensory nerve with its cell bodies in the nodose ganglion. In the region of the nodose ganglion the vagus trunk is independant of the spinal accessory nerve with which it is fused proximally.

The nodose ganglion and the vagus trunk in the neck are wrapped, with connective tissue, together with the superior cervical ganglion and trunk. However, no interchange of fibers takes place between the nerves (44).

The nerve below the ganglia

Agostini et al (2) have conducted functional and histological studies of the vagus and its branches to the heart, lungs, and abdominal viscera in the cat. They counted about 30,000 fibers in the cervical vagus, 16% of which are unmyelinated. Afferents constitute 20% of the total and their cell bodies are in the vagal ganglia. The abdominal vagus consists largely of unmyelinated afferent fibers.

The aortic nerve, which is a branch of the thoracic vagus in most species, contains about 450 fibers, two-thirds of which are myelinated in the cat (34). The myelinated fibers have a bimodal distribution with peaks at 2 - 4 μ and 8 - 10 μ diameter. Douglas, Ritchie and Schaumann (19) conclude from action potential studies that the aortic nerve contains large depressor fibers, small pressor fibers, and small depressor fibers. This observation is consistent with the work of Landgren (46) which implies the existence of two functional types of pressoreceptors. De Burgh Daly and Evans (16) have described myelinated fibers of the 2 - 4 μ diameter group and non-myelinated fibers serving the motor functions of the heart. The afferent functions are served by myelinated fibers found in all diameter groups (1 - 14 μ) and by non-myelinated fibers.

The conduction velocities of respiratory and cardiovascular afferent fibers in the vagus have been measured by Paintal (60). These vary from 6 to 36 metres/sec. Paintal mentions some correlation between the amplitude of the action potential and the fiber size but he also points out exceptions to this rule.

The nodose ganglion

Jones (44) says that all the cells of the nodose ganglion are unipolar and no evidence of synapses exists. The cell-fiber ratio and the excess of fibers distal to the ganglion over those proximal to it strongly suggest to him, however, that

some of its cells are motor. De Burgh Daly and Evans (16) deny the existence of motor cells in the nodose ganglion. The evidence of Ranson (50) that many fibers become smaller and more difficult to stain after passing through the nodose ganglion would explain the observation of Jones. Many of the cells in the nodose ganglion are of the fenestrated type (68).

Since this review was written, a paper by Bonvallet and Sigg (9) has come to light in which the distribution of cardiac and pulmonary afferent fibers in the vagal rootlets is described. Owing to the direct bearing which this paper has on the present study mention of it is made here, however, discussion of the paper is reserved until later.

The vagus nerve above the ganglia

On the assumption that the jugular and the nodose ganglia are sensory, Du Bois and Foley (20) have described the sensory and motor components of the vagus on the basis of degeneration studies. They describe the separation of these components in the rootlets. The sensory component occupies, largely, the most dorsal and cephalad portion of the rootlets, while the motor component is ventral and caudad (24).

Tarlov has reached similar conclusions to Du Bois and Foley but has used a different technique. The glial covering on the sensory rootlets is longer than on the motor rootlets and "cell piling" is more prominent at the transition zone on the sensory rootlets (78). Tarlov notes that the vagus-spinal

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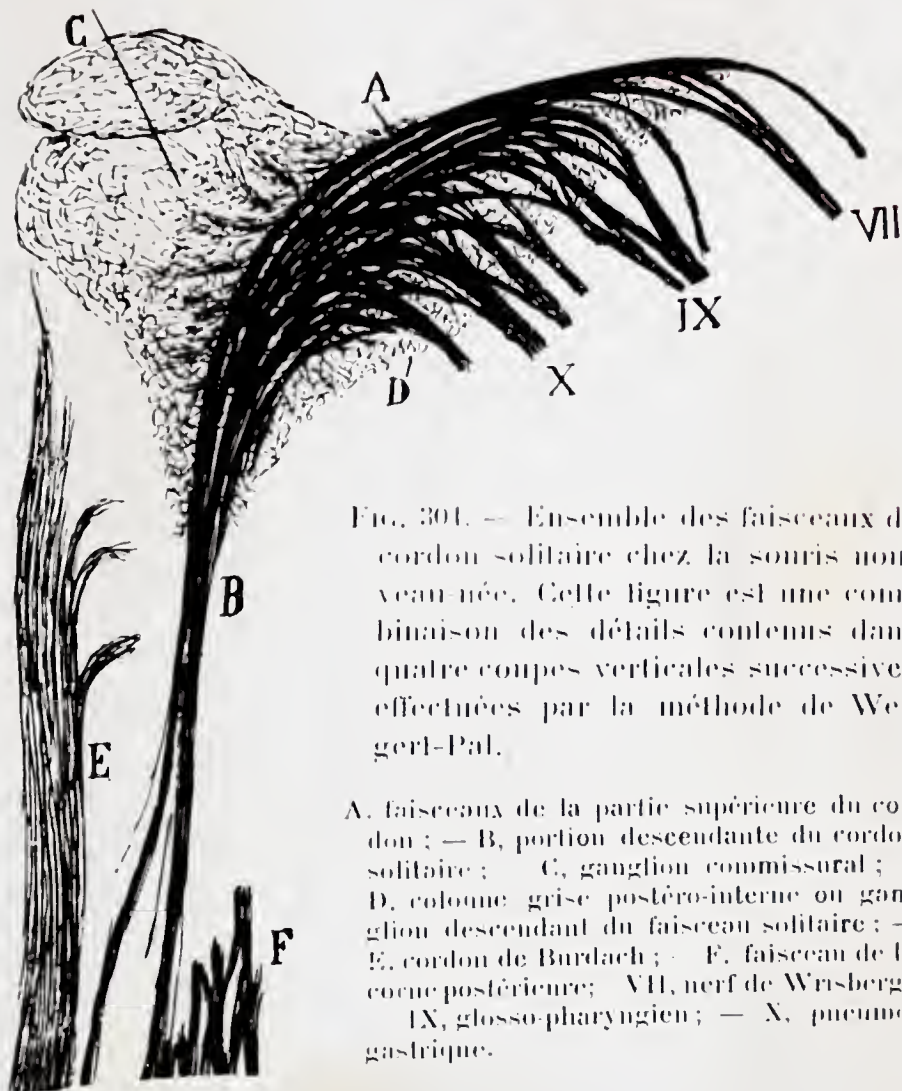


FIG. 301. — Ensemble des faisceaux du cordon solitaire chez la souris non-veau-née. Cette figure est une combinaison des détails contenus dans quatre coupes verticales successives effectuées par la méthode de Weigert-Pal.

A, faisceaux de la partie supérieure du cordon ; — B, portion descendante du cordon solitaire ; — C, ganglion commissural ; D, colonne grise postéro-interne ou ganglion descendant du faisceau solitaire ; — E, cordon de Burdach ; — F, faisceau de la cône postérieure ; — VII, nerf de Wrisberg ; IX, glosso-pharyngien ; — X, pneumo-gastrique.



Fig. 4

Diagram of the descending course of the tractus solitarius. Fibre tracts only are shown. (From Cajal, S. R. Histologie du systeme nerveux. Paris, 1907).

accessory complex often consists of two distinct motor portions, one contained in the small ventromedial roots (c.f. Fig. 3), and the other in the large caudal roots. "The cephalic portion of this complex is largely sensory" (78).

The tractus Solitarius

The developmental anatomy of the afferent systems to the medulla have been described by Kappers, Huber, and Crosby (45).

The seventh, ninth and tenth nerves send their sensory component to the tractus solitarius. Cajal states that all the vagal fibers turn caudalwards (12). Foley and Du Bois have noted from degeneration studies that three types of vagal rootlets are found passing into the tractus solitarius in the medulla. The three types are the same as those found in the extramedullary rootlets: a pure sensory type, a pure motor type, and a mixed sensory-motor type.

Beginning at the level of entrance of the most caudal vagal sensory rootlets the tractus solitarius gives off collaterals to a medial nucleus. Just above the obex collaterals pass to a circumferential nucleus. Below the obex collaterals enter the medial nucleus of Cajal, (12). Three fourths of the tract crosses in the commissura inferna to end in the nucleus of Cajal. The remaining fourth descends into the cervical cord (45).

The increase in the post vagal portion of the tractus solitarius and its associated grey is associated with the increase in the general visceral sensory components of the glossopharyngeal

and vagus nerves. The development of the post vagal tractus first clearly becomes evident in those animals in which respiration through the lungs substitutes for gill breathing. "In some animals the fasciculus solitarius has been seen to descend into the cervical cord, and secondary descending neurons arising from the grey associated with this fasciculus have been traced at least as far caudalward as the fourth cervical segment." (45).

Not all vagal fibers enter the tractus solitarius. The somatic afferent fibers carrying pain and tactile sensibility separate from the visceral afferent fibers and become mixed with the descending root of the trigeminal nerve eventually ending in its nucleus. Kappers et al regard this as a good example of the reorganization of fibers within the medulla into patterns governed by functional relations of the components rather than by their peripheral courses.

Anderson and Berry (4) have recorded evoked potentials in the tractus solitarius by stimulating the vagus nerve and its branches. Responses to stimulation of the aortic depressor nerve were recorded in the tractus solitarius and the sensory nucleus of the vagus. Stimulation of the vagus distal to its superior laryngeal branch evoked activity in the tractus solitarius and the ipsi and contralateral half of the vagal commissure. Some features of the compound action potential and the increasing complexity of the action potential recorded from the tractus solitarius as it descends toward the vagal commissure lead these authors to two

conclusions.

1. Primary vagal fibers reach the opposite commissure and nucleus.

2. A large part of the activity following the primary volley in the caudal tractus solitarius represents post synaptic activity. Hence both primary and secondary fibers reach the contralateral nucleus.

An objection to this method of studying central nervous phenomena is that the method of stimulation is not very physiological and has the disadvantage that the compound action potential represents activity in many fibers not normally stimulated at the same time. This effect is liable to lead to the opening of synaptic pathways that do not operate in the normal animal.

The nucleus solitarius

Olszewski and Baxter in a recently published atlas (57) have divided the nucleus solitarius into three parts.

1. A portion dorsal to the tractus solitarius composed of small fusiform cells.

2. An area of larger more densely staining cells surrounding the tractus solitarius.

3. Cells similar to the cells of the dorsal motor nucleus situated among the cells of the nucleus solitarius. There are also always found some cells among the fibers of the tractus solitarius itself.

This division is indeed interesting in view of Olszewski's

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contention that cells of differing morphological types must differ also in some functional way.

The caudal nucleus is believed to be entirely vagal in its connections.

Herrick (33) has shown the existence of ascending fibers in the brain stem of the lamprey which are derived from the nucleus solitarius and from the nucleus of Cajal, which may be regarded as the caudal end of each of the solitary nuclei. These fibers have been seen to ascend in the lateral reticular formation to the mesencephalon and as far as the hypothalamus.

Brodal, Szabo, and Torvik have presented anatomical evidence for the function of the nucleus solitarius as an integrative centre (11). Fibers arising in the cerebral cortex terminate in all parts of the nucleus solitarius. It has also been found that a small number of ascending fibers from the spinal cord terminate in the nucleus. Torvik points out that exact knowledge of the connections from the reticular formation to the nucleus solitarius is lacking but that if such connections exist then the solitario-spinal fibers could be concerned with the transmission of information from medullary centres to the effector organs.

In view of the functional connection between the afferent vagal signals and the vital centers of respiration and vasomotor control in the brain stem the location of these vital centers will be briefly reviewed in the next section.

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Vital centers in the brain stem

Ludwig and Cyon first discovered reflex control of the cardiovascular system in 1866 when they produced bradycardia and hypotension by stimulating the central end of the aortic depressor nerve. However, it was Ludwig's pupil Dittmar who in 1870 localized a vasomotor center in the medulla by observing the onset of hypotension as the brain was sectioned from before backwards. Since that time the reticular formation has been increasingly implicated in vasomotor control.

Many workers, notably Ranson and Billingsley and Wang (49, 73, 80), have contributed to producing the following facts. The pressor areas of the brain stem are located in the medulla near the facial colliculus and at the inferior fovea on the floor of the fourth ventricle. The lateral reticular formation and the periventricular gray matter and the tegmentum of the pons are also involved in pressor response.

Depressor areas of the brain stem have been found in the area postrema, the caudal dorsal medulla, and the lateral and medial reticular formation. In the pons the depressor area is situated in the ventrolateral tegmentum.

Generally there seems to be a lateral pressor area and a medial non-specific depressor area which inhibits vasomotor tone. Lateral specific vaso-inhibitory neurons can be found ().

In the 19th century many workers localized areas for respiratory control in the medulla. Mannier and Pitts et al

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(53, 69, 70, 71) at the beginning of this century localized areas in the reticular formation which control expiration and inspiration. The respiratory center as a whole is located caudal to the entrance of the eighth cranial nerve and dorsal to the inferior olivary nuclei. The inspiratory area is located in the medial reticular formation and the expiratory area in the dorsolateral reticular formation.

An area further rostral discovered by Lumsden (50) and called the pneumotaxic center has an inhibitory action on the respiratory centers.

Many workers (3, 7, 18, 51) have observed mixed effects on stimulating areas of the reticular formation. Sometimes vasomotor and respiratory effects occur together. Allen in 1932 first stressed the polyfunctional properties of the brain stem reticular formation, and recently Bach (8) has studied the reticular formation and has shown changing degrees of mixed function depending on the area stimulated.

Generally it would seem that if all neurones are regarded as being divided into sets, then the set of neurones having a cardiovascular function overlaps the set of neurones having a respiratory function. Whether the number of elements in each set is constant through time and whether the common area remains the same is not known.

A short history of the methodology of central nervous system
investigation

The beginnings of a methodology in the physiological investigation of brain function were made during the 17th century. The method was that of stimulation and ablation of the nervous tissue.

In this early period of neurophysiology both stimulation and ablation were mechanical and ablation usually occurred as a necessary consequence of stimulation. These procedures led to observations of behaviour and theories of the sites of vital functions. Willis in 1684 proposed that the cerebellum was the center of vital functions because his animals died on cerebellar ablation. However in 1760 Lorry showed that the death had actually been due to Willis' faulty technique and proposed that the medulla was the center of vital functions because when he pushed a probe into it his animals responded by going into convulsions. This is much the same observation that Herophilus made in 300 B. C.

Now a transition period took place. Discoveries of electrical forces led Galvani in 1791 to describe what he called animal electricity. Three years later Galvani performed the experiment which he called "contraction without metals" -an experiment in which the injury potential of a muscle stimulated a second nerve-muscle preparation. This experiment heralded the discovery of the action potential fifty years later by Matteueci and Du Bois-Reymond, and, following this, the application of recording

THE UNITED STATES OF AMERICA

OFFICE OF THE SECRETARY

WASHINGTON, D. C.

DEPARTMENT OF THE INTERIOR

BUREAU OF LAND MANAGEMENT

1910

TO THE SECRETARY OF THE INTERIOR

FROM THE CHIEF OF BUREAU

SUBJECT: LANDS OF THE UNITED STATES

IN RESPONSE TO YOUR LETTER OF

THE 10TH INSTANT, RELATIVE TO

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techniques to the central nervous system.

During the transition period from techniques of mechanical stimulation to those of electrical stimulation the methods were gross. A Voltaic pile was used and the usual result was widespread twitchings and convulsions of the animal. Aldini even stimulated the severed heads of criminals by this method. His descriptions of the facial contortions of his specimens leads one to suspect that he was troubled experimentally by direct stimulation of the facial muscles by the spreading stimulating current.

The modern era of stimulation and ablation was initiated when Ruhmkorff invented the induction coil. This enabled more localized studies to be made because the output of the coils was adjustable. The general method of this early period of the modern era was to study the motor functions of nervous tissue by stimulation and the sensory functions by ablation.

The method of localized stimulation followed by controlled ablation was a powerful one and its influence still colors our concepts of neurological organization. The concept to which it gave rise was that of localization of function. This concept was strengthened by the development of sensitive psychological methods of testing for specific disabilities. However, the basic concepts are still of doubtful logical nature.

The unproved assumptions are:

1. That if the destruction of a given anatomical locus in the nervous system results in a specific functional deficit --

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the function in question resides in the locus.

2. That a given locus has a function which it exercises regardless of the biography of the organism, the object of its activities, the stimulus arrangements, and the conditions of internal and external environment (82).

That these assumptions are not proven has not stopped them being of use, but it is just as well to emphasize their status.

The development of a recording technique to study the electrical activity of nervous tissue had to await the invention of the vacuum tube. In 1923 the cathode ray oscilloscope was first used to visualize the action potential of the nerve. Recording was effected with the use of small bipolar or monopolar electrodes until just after the last war when Ling and Gerard at the University of Chicago produced micropipettes with a tip diameter of half a micron. These electrodes can record from the inside of a nerve cell. This development has focussed much modern interest on the physiology of single nerve cells. It remains to be seen whether the function of the whole brain can be explained in terms of the behaviour of its individual members.

INSTRUMENTATION

The experimental work consisted of recording single unit action potentials from the peripheral and central nervous systems using microelectrodes. Methods of making glass and tungsten microelectrodes were investigated together with methods for measuring their resistances.

The experimental animal was the cat anaesthetized with nembutal and immobilized with a Johnson stereotaxic machine. The recording equipment was constructed from standard commercial units.

The Stereotaxic Machine

A standard Johnson stereotaxic machine was used to immobilize the cat. The electrode carrying head supplied with the machine was used but was found, late in this series of experiments, to be of insufficient rigidity for microelectrode recording. Under a dissecting microscope, a circular motion of the electrode tip could be seen as the screw of the micrometer advance was turned. This circular motion was caused by "whip" in the system.

The lateral movement of the electrode tip leads to two undesirable results:

1. The electrode tip breaks.
2. The nerve cells are unnecessarily damaged.

For these reasons, it is recommended that a different system for advancing the electrode be used in future experiments of this series. An hydraulic advancing device could be used.

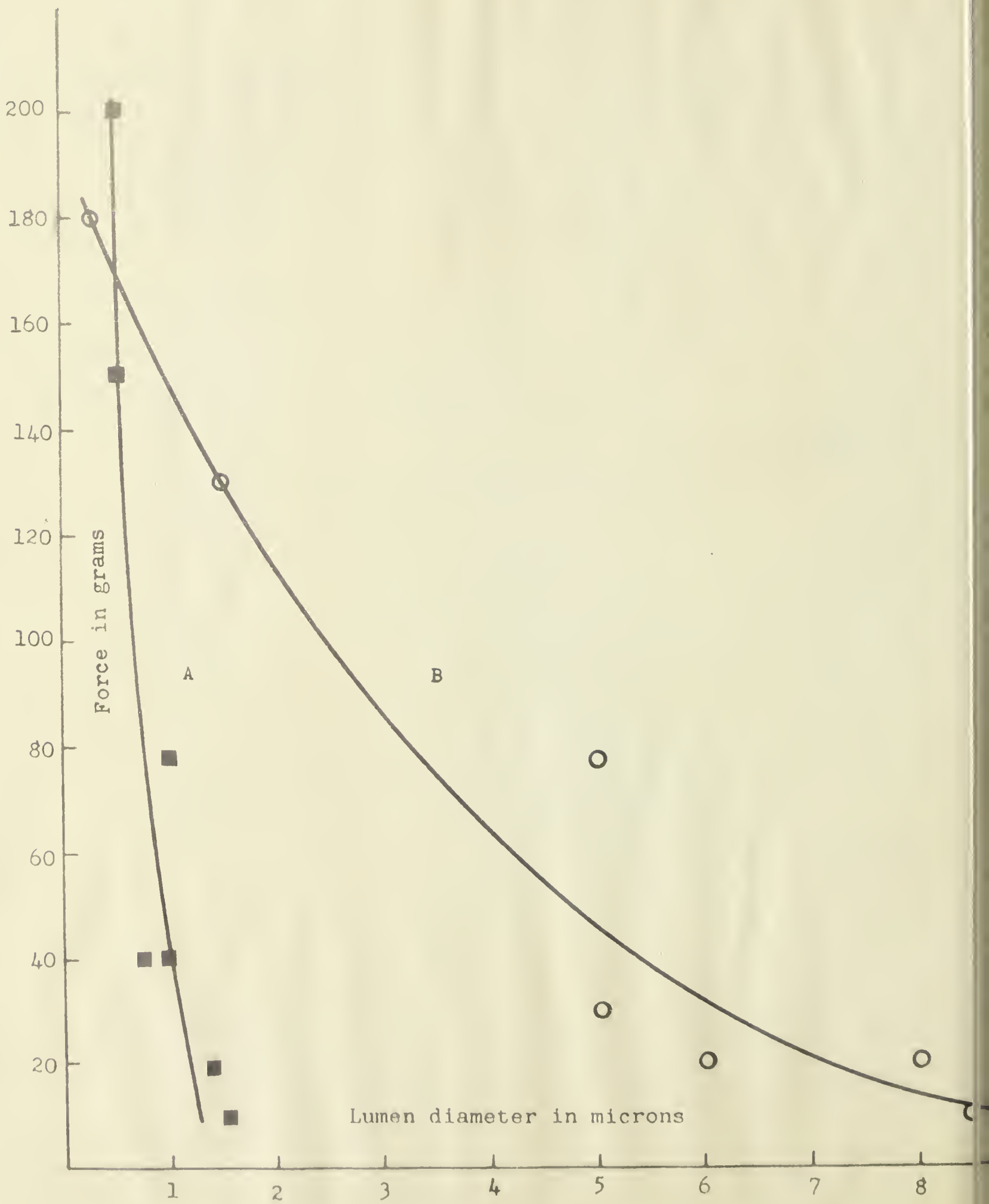


Fig. 5

Force-lumen diameter diagram for 1 m.m. external diameter soft glass tubing pulled at heating coil voltages of 7 V (Curve A) and 8 V (Curve B). Each point shown on the diagram represents a mean value of four observations. The curves were fitted to the points by eye.

Microelectrodes

Tungsten microelectrodes as described by Hubel (39) were made but not used experimentally for the following reasons:

1. The resistance was generally too high.
2. The electrodes are polarizable, hence the voltage transmitted depended on the pH at the tip (17).
3. The application of an insulating coat to the electrode was difficult to control.

In contrast, glass microelectrodes were found to be easy and quick to make and were also reproducible in tip diameter and taper.

In the manufacture of glass micropipettes, glass tubing of 1 mm external diameter was used. Preliminary tests were performed to determine the properties of this glass when pulled with various forces at various temperatures. These tests were made on similar lengths of glass hung vertically from a clamp. A nichrome wire coil, 5 mm in diameter, was used to heat the glass at its midpoint. The resistance of the nichrome wire was 0.08Ω per cm. length. Current to the coil was controlled using a variac transformer and the voltage drop across the coil was measured using an A. C. voltmeter. The pulling force on the glass was supplied by weights hung from the lower end of the tubing.

Figure 5 summarizes the results of these tests. Curve A was produced when a voltage drop of 7 v. occurred across the coil. The electrodes in this case had rather abrupt tapers. Curve B was

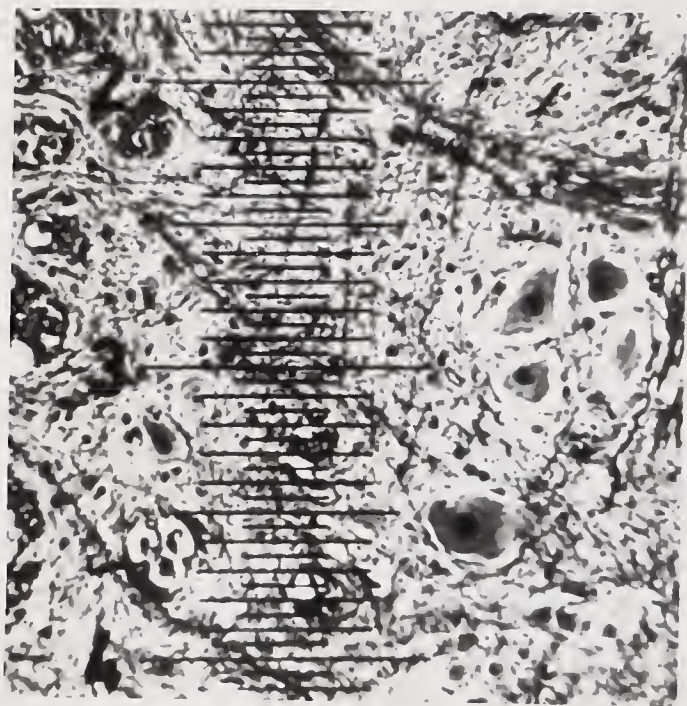


Fig. 6

Composite photomicrograph showing microelectrodes at extreme tip diameters of the range produced. The cells shown at the left are from the dorsal motor nucleus of the vagus and are stained with Weil's stain. Scale: one smallest division equals 7.5 u.

produced at a voltage drop of 8 v. across the coil, the electrodes in this case having more gradual tapers.

With this information in mind, a mechanical electrode puller was built after the design of Winsbury (83). A circuit diagram of this machine is shown in Figure 9, and its operation is as follows:

1. The glass tubing is threaded through the heating coil and clamped in a vertical position in rubber jawed holders.
2. The on-off switch is turned on.
3. The heater switch SW1 is turned on.
4. Events now occur automatically. The coil heats and the glass tubing extends under the 100 gm. weight of the lower clamp which is attached to the solenoid plunger. After about 1 cm. extension, the microswitch SW2 is tripped and the solenoid becomes activated, exerting a force of 2,000 gm. on the glass tubing. The heater coil is immediately turned off as microswitch SW3 is tripped. The excursion of the solenoid plunger is stopped by contact with the face of the solenoid. Bounce is prevented by the solenoid remaining on until the on-off switch is turned off.

This machine was improvized rather than designed and could be extensively improved. However, it quickly produced suitable glass micropipettes in a reproducible manner. Two electrodes are illustrated in Figure 6.

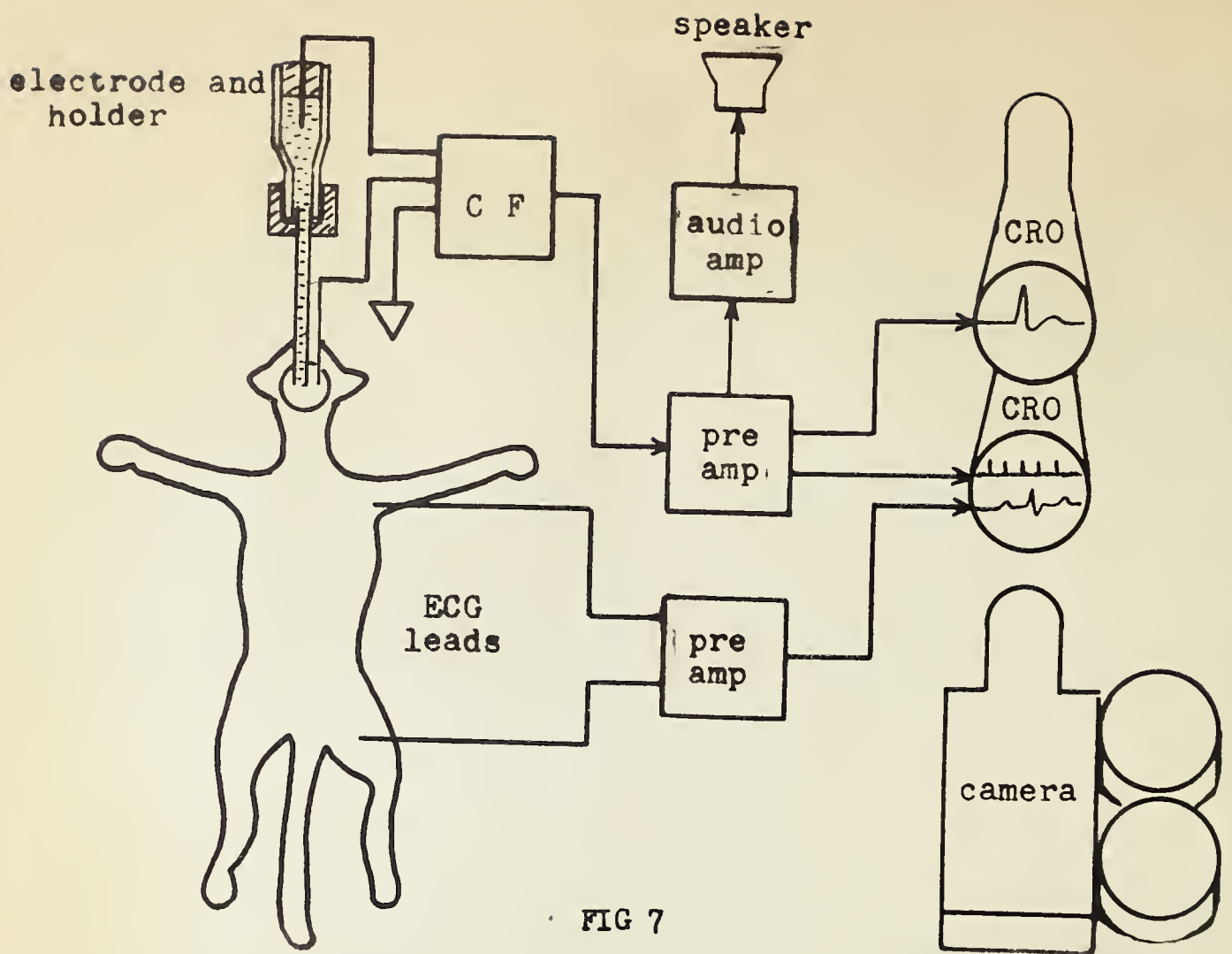


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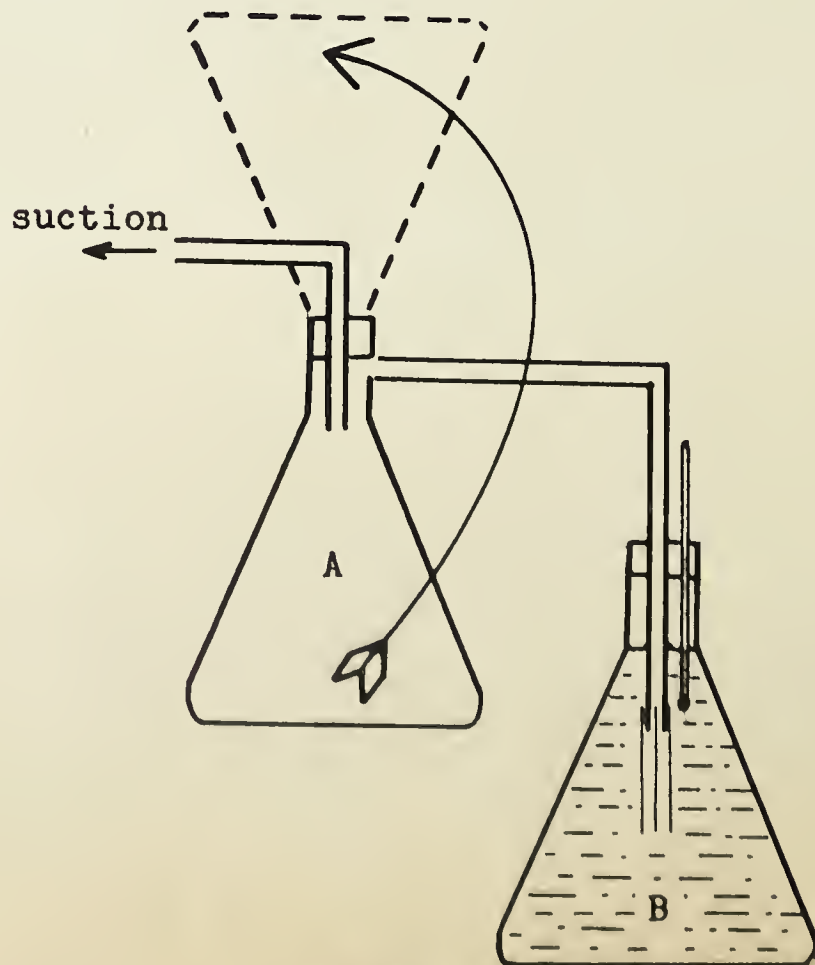


FIG 8

Fig. 7

Block diagram of the arrangement of the apparatus.

CF, cathode follower; CRO, cathode ray oscilloscope;
amp, amplifier.

Fig. 8

Diagram of the apparatus used for filling the micro-electrodes with a salt solution. See text for explanation.

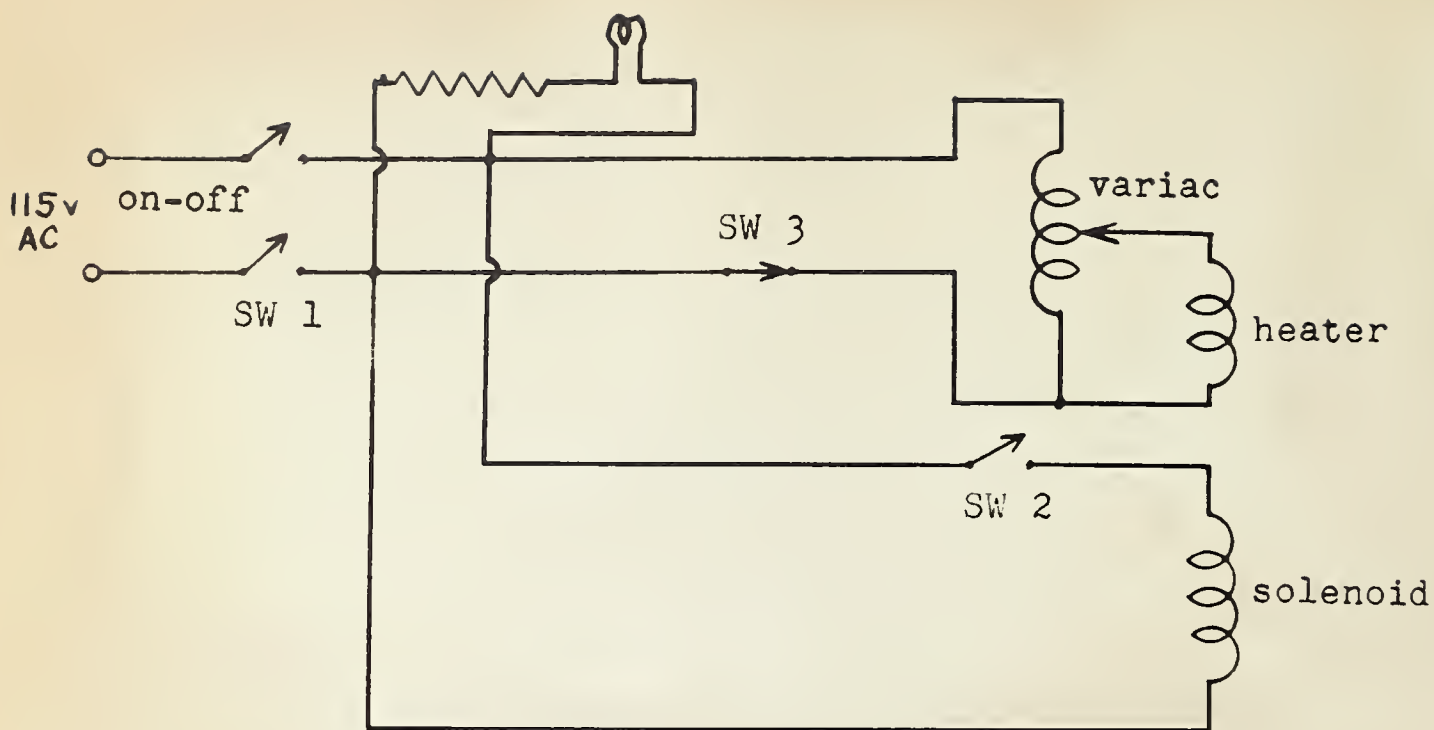


FIG 9

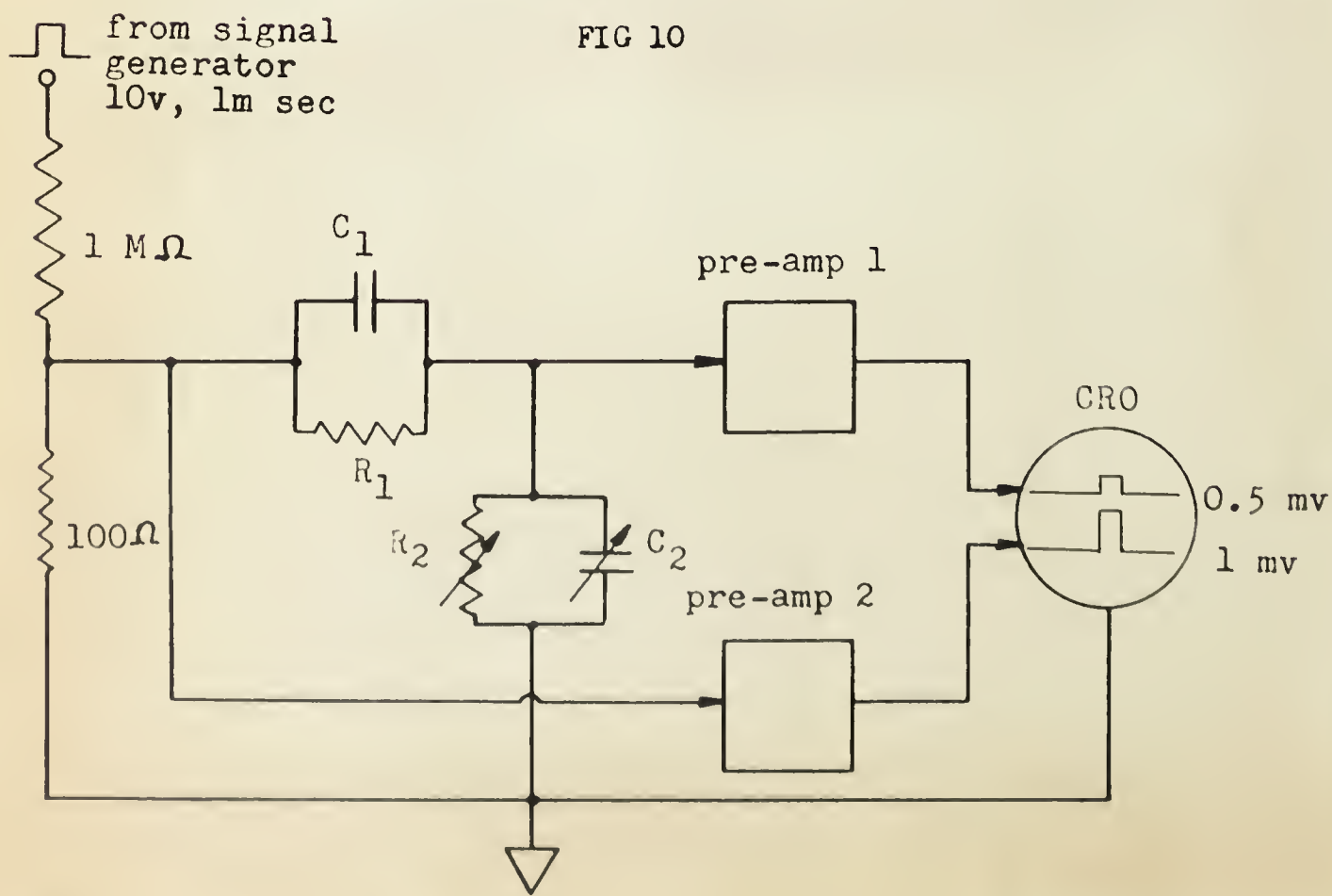


FIG 10

Fig. 9

Circuit diagram of the electrode pulling machine.

SW1, SW2, SW3, are switches.

Fig. 10

Circuit diagram of a device used to measure the resistance and capacitance of electrodes. Explanation in text.

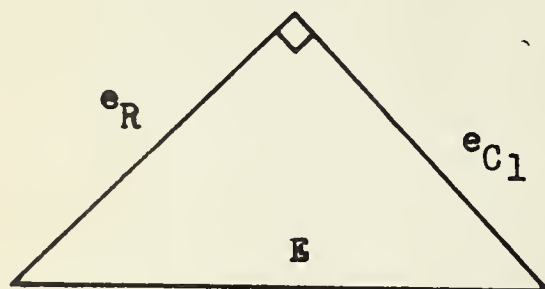
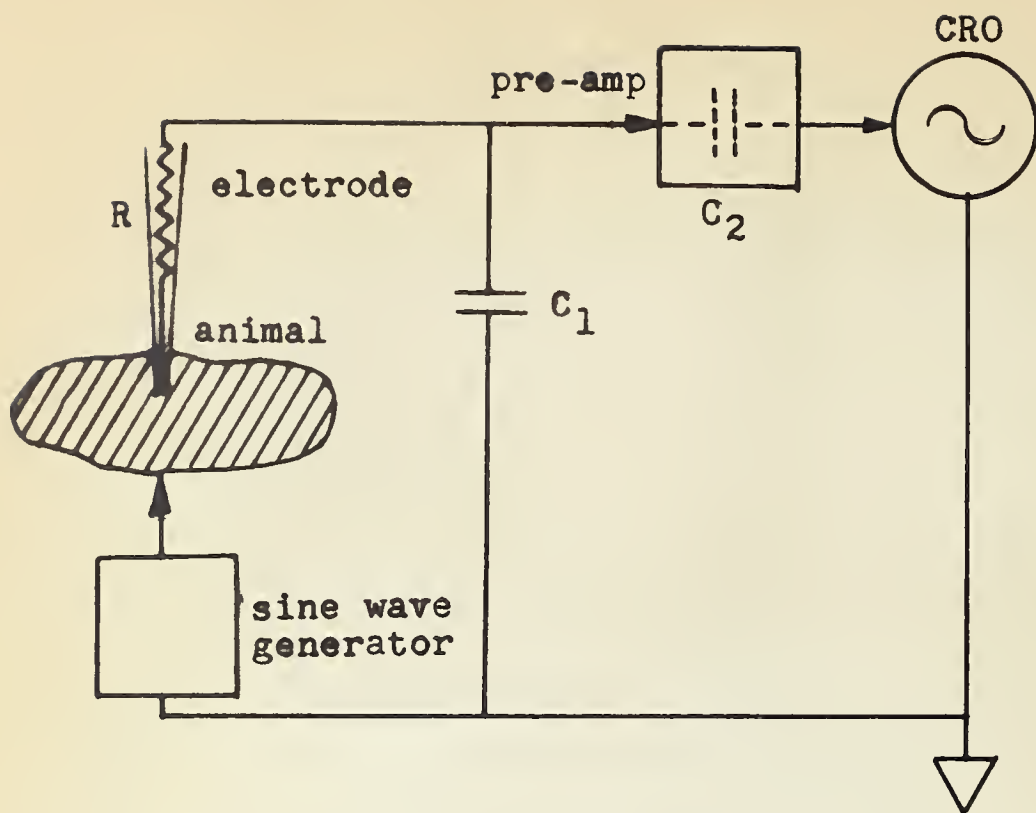
Filling the pipettes

The micropipettes, being intended to record extracellularly, were of fairly large tip diameter, of from 2 - 20 μ . This being the case, the electrodes were filled with isotonic Ringers solutions, because KCl leaking from the large tip would damage the nerve cells. The electrodes were filled under reduced pressure using the apparatus diagrammed in Figure 8. Flask A contained the Ringers solution which was heated to about 70° C, The electrodes were suspended, tip down, around a centrally placed rod. The Ringers solution was boiled under reduced pressure for 10 minutes during which time water collected in Flask B. When at the end of 10 minutes the interior of the system was returned to atmospheric pressure, Flask B. was inverted allowing liquid in it to be forced back into Flask A. In this way the electrodes were kept immersed and the Ringers solution was kept at approximately the same concentration.

Resistance of the Electrodes

Rough testing of the resistance of the electrodes was effected using an ohmmeter and a bath of Ringers solution. This method sufficed to give the order of resistance of the electrodes and enabled defective electrodes to be quickly discarded.

A circuit was built to measure both the resistance and the capacitance of the electrodes. The circuit is shown in Figure 10. C_1 and R_1 represent the values for the capacitance and



R is the electrode resistance

E is the electrode input voltage

e_R is the IR drop across R

e_{C1} is the IR drop across C_1 at high input frequencies.

C_1 and C_2 are capacitances.

If $C_1 \gg C_2$ then $E = e_R + e_{C1}$

e_{C1} lags e_R by 90°

then $e_R = \frac{1}{\sqrt{2}} E$

and $e_R = 0.707E$

Fig. 11

Circuit diagram and theoretical explanation of a device used to measure the resistance of an electrode while in a recording position.

resistance of the micropipette. The variable resistance R_2 and the capacitor C_2 are adjusted to give an undistorted signal from pre-amplifier 1 which is exactly half the amplitude of the signal fed directly to pre-amplifier 2. In this "matched" condition, $C_1 = C_2$ and $R_1 = R_2$. The circuit was the suggestion of Kent M. Chapman.

A circuit for measuring the resistance of the micro-electrode while in a recording position in the animal's brain was also built after the description of Skoglund (76). Figure 11 shows a schematic diagram of the circuit together with the electrical theory leading to a measurement of the electrode resistance R from the frequency at which the amplitude of a recorded sine wave dropped to 70% of its value at low frequencies (20 c/sec). A factor which affected the usefulness of this technique was the difficulty experienced in holding the sine wave pattern on the oscilloscope screen while the medulla was undergoing slight shifts due to respiration and the vascular pressure pulse. However, this method should be of considerable value in checking for pipette breakage if the recording field can be stabilized.

RECORDING APPARATUS

All the nerve potentials were measured by coupling the electrode through a high impedance probe to a capacity coupled A. C. amplifier and leading the amplified signal into a cathode ray oscilloscope.

The cathode follower unit was the high impedance probe made by the Grass Instrument Company and intended for use with the model P5 preamplifier. The output impedance was about 4,000 Ω and the grid current was rated at less than 10^{-9} amperes.

Leads from the microelectrode and from the indifferent electrode on the cat were made from short lengths of bare copper wire to minimize stray capacitance effects. Connections to the saline of the microelectrode and to the flesh of the cat were made with non-polarizable Ag-AgCl wires. The ground connection was made to the stereotaxic machine.

A. C. amplification of the output of the cathode follower was effected with the Grass P5 amplifier operated with a push-pull input and double ended output at a band width of from 35c/sec to 10Kc/sec. The "peak to peak" noise level of the amplifier was 10 microvolts which was negligible compared with the noise of the electrodes. (Figure 25).

Two oscilloscopes made it possible to both see and photograph the nerve action spikes. The monitor oscilloscope was a Heath Kit laboratory model. The oscilloscope used for photography was a double beam Nagard unit type 103. Nerve action

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8. The eighth part of the report is devoted to a detailed analysis of the conclusions and recommendations of the report.

spikes were displayed on one beam of the oscilloscope and the electrocardiogram of the animal was displayed on the other after having been amplified by the Nagard 2701 pre-amplifier. Voltage calibration pulses could be fed from the Grass pre-amplifier into the channel used to display nerve impulses.

A Dumont oscillograph record camera type 321-A was used to photograph the display on the Nagard oscilloscope. No time base was used on the oscilloscope during photography because the motion of the film acted as a time base. A neon light in the camera supplied 120 cycle/sec timing marks on the film in the form of spots at the bottom of the film.

All the apparatus was shielded from the A. C. power supply and from other sources of stray interferences by recording inside a copper wire mesh room that was grounded to the water pipes in the laboratory.

METHODS

Forty-two cats were used in this investigation, 20 for attempts at recording peripherally in the nodose ganglion, vagus trunk and vagal rootlets and 22 for central nervous system recording. Except where specifically stated, recording was accomplished using glass micropipettes extracellularly. The animals were anaesthetized with sodium pentobarbitone, 35 mgm/Kg given intraperitoneally; if more anaesthesia was required later in the experiment, intravenous sodium pentobarbitone in saline was given.

Nembutal was thought to be a suitable anaesthetic for this series of experiments because it has been shown not to affect the initiation and propagation of impulses in the nerve up to at least the first synapse. As the initial object of this investigation was an attempt to record the primary afferent groups of nerve impulses within and outside the central nervous system, the possibility of blockage of the impulses at the first or subsequent synapses was not an object of concern. Indeed, it was thought that blockage of activity within the reticular formation would yield a quieter background for recording. It is likely that cardiovascular and respiratory pathways are not interrupted by light anaesthesia, and only modified during deeper anaesthesia.

(A) Recording in the Peripheral Nerve

(a) Nodose ganglion and vagus trunk

A midline incision was made on the ventral side of

the neck for a length of about 7-8 cm. The neck muscles were separated to expose the trachea and a tracheotomy tube was tied into the trachea. To facilitate recording from the nerve with a micropipette, the animal was fixed on its back in the stereotaxic machine. The pipette was lowered into the nerve through an incision in its connective tissue sheath. In one experiment the aortic depressor nerve was separated on the left side of the neck and positively identified by recording from it without dissection of the nerve into fibers. Four experiments were performed in which the nodose ganglion and vagus nerve distal to the ganglion were entered.

(b) The vagal rootlets

The approach to the vagal rootlets was made on the left side of the head. A midline skin incision was made at the posterior half of the skull and from the mid-point of this incision another cut, normal to the first, was made down to the posterior edge of the left pinna. The triangular skin flaps were reflected. The temporal muscle was divided at its attachment to the skull and reflected laterally. A trephine hole was made close to the superior nuchal ridge and centered about 2 cm. from the midline. If necessary, the trephine hole was slightly enlarged so that the left side of the cerebellum could be seen. Bone bleeding was controlled with plasticine and pieces of fresh muscle. A star-shaped incision was made in the dura covering the cerebellum and



Fig. 12

Photograph of dorsal view of cat's medulla as seen after the occipital bone and cerebellum have been removed.

the cerebellum was removed by suction until the vagal rootlets could be seen when looking down through the trephine hole.

In several experiments very fine bipolar "pick-up" electrodes made of silver were used in an attempt to record from individual rootlets. Blood was removed from the field during these experiments by using either cotton swabs or suction applied through fine polythene tubing, the mineral oil level being continuously replenished by a constant drip. The use of pick-up electrodes eventually seemed to be impractical and an attempt to record from the rootlets was made using micropipettes. In both cases the animal was immobilized in the Johnson stereotaxic machine.

(B) Recording in the Central Nervous System

Twenty-two cats were used in the investigation of intramedullary recording. The anaesthetized animal was immobilized in the stereotaxic machine before the operating was performed. A midline skin incision over the posterior half of the skull was intersected by a transverse incision extending across the back of the skull behind the ears. The four skin flaps formed were reflected allowing the temporal muscles and the muscles of the neck to be separated from the skull. The atlanto-occipital membrane and dura were cut in a transverse direction and the occipital bone was removed bilaterally up to the bony tentorium. This procedure left the posterior medulla and the cerebellum exposed. A major part of the cerebellum was removed by suction

leaving the floor of the fourth ventricle exposed. (See Fig. 12).

At this stage the electrode, mounted in the Johnson manipulator, could be fixed to the stereotaxic machine and lowered into a recording position. The obex was used as a coordinate reference point and all subsequent measurements were made relative to the measured position of the obex. The surface level of the medulla was signalled by a bang in the loudspeaker when the electrode tip made contact with the medulla. Below the surface the electrode was advanced slowly, its progress being monitored continuously by ear from the loudspeaker and by eye from the oscilloscope pattern. Activity of interest was photographed from the oscilloscope screen and the depth of the tip of the needle was noted in microns. Electrodes selected for central recording were of external tip diameters from 2- 20 microns. The resistance of these electrodes varied between 5.0 and 0-5 megohms.

After the experiment the head of the animal was perfused with isotonic saline followed by 10% formalin. The brain was left in situ overnight and in the morning was removed and placed in 10% formalin for storage. Needle positions were checked by the technique described by Fox and Eichman (86) the brain stem being sectioned on the freezing microtome at 100 μ . The brain stem was sectioned serially in paraffin wax in the case of one animal only. These sections were stained using Weil's method.

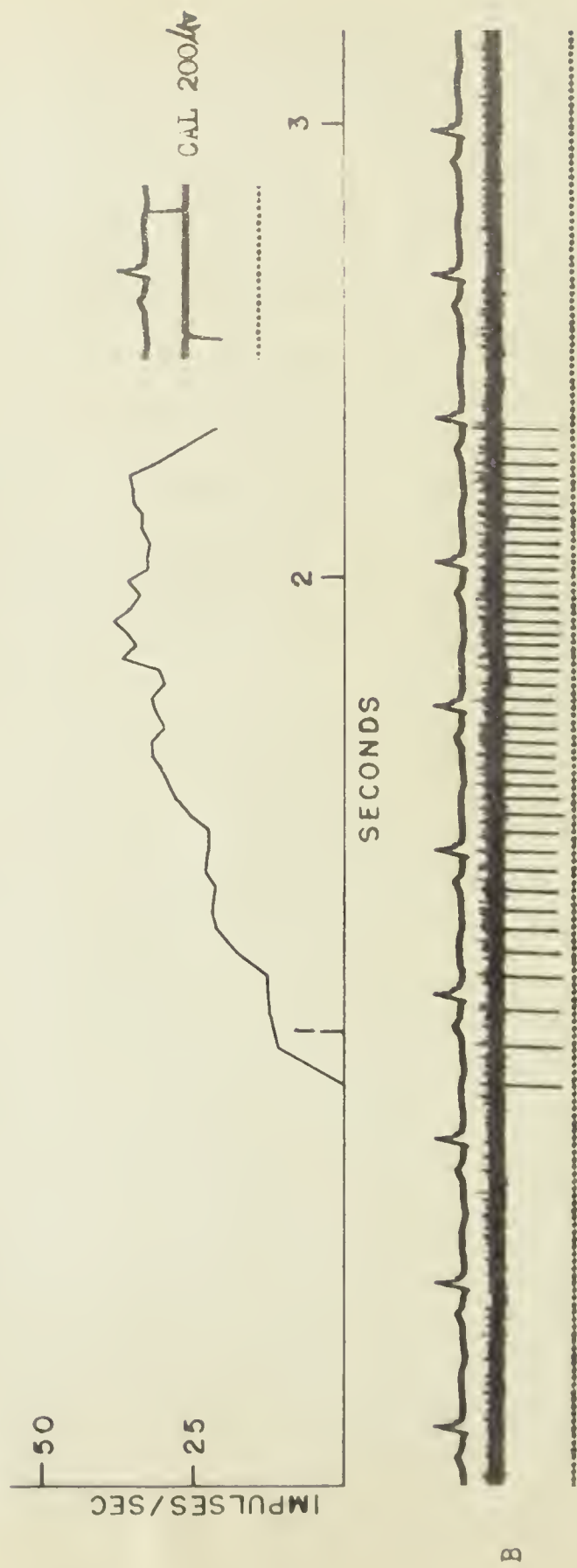
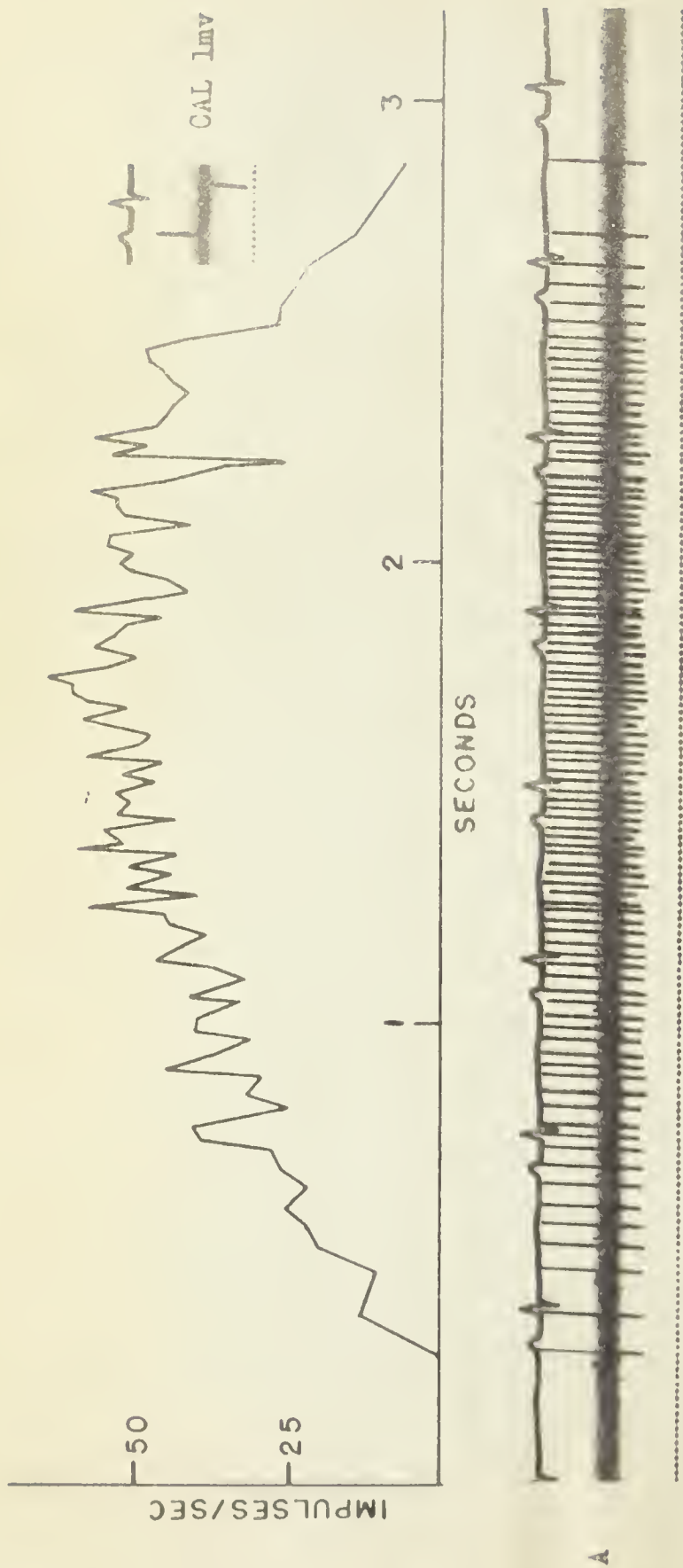


Fig. 13

A. Inspiratory discharge from respiratory center at position K3 of Fig. 15 and depth 3.75 mm.

The frequency plot is uppermost; the record traces from top downwards: ECG, nerve activity with impulses retouched, time marker 60/sec.

B. Inspiratory discharge from left nodose ganglion.

Frequency plot uppermost; the record traces from top downward: ECG, nerve activity with impulses retouched, time marker 60/sec.

A



B

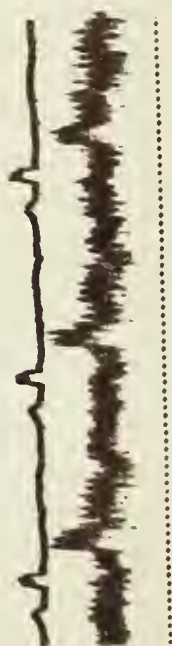


Fig. 14

A. Inspiratory discharge from left nodose ganglion with a superimposed cardiac rhythm. Record traces from top downward: ECG, nerve discharge with nerve spikes retouched, 60/sec time marker.

B. Compound nerve activity recorded with a micropipette from the whole left aortic depressor nerve under saline. Record traces from top downward: ECG, nerve activity, 60/sec time marker.

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RESULTS

(A) The peripheral vagus nerve

Recording in the peripheral nerve with microelectrodes yielded analyzable results only in the nodose ganglion where respiratory discharges were picked up (Figs. 13B and 14A). Respiratory discharges similar to those shown in Fig. 13B were also recorded in the nerve trunk about a centimeter distal to the nodose ganglion. The marked absence of recordings from nerves having a cardiovascular discharge led to an experiment in which the aortic depressor nerve on the left side was positively identified with a pair of silver pick-up electrodes. A microelectrode was then butted against the nerve which was covered with its connective tissue sheath. In this condition the record shown in Fig. 14B was obtained. The trace showing the potential of the nerve sheath at the point of application of the microelectrode shows a deflection in each cardiac cycle. However, when the nerve sheath was split open under saline and the microelectrode tip inserted into the nerve, no deflection occurred, and no recording of any recognizable type of nerve activity was made. The possible significance of these results is discussed later.

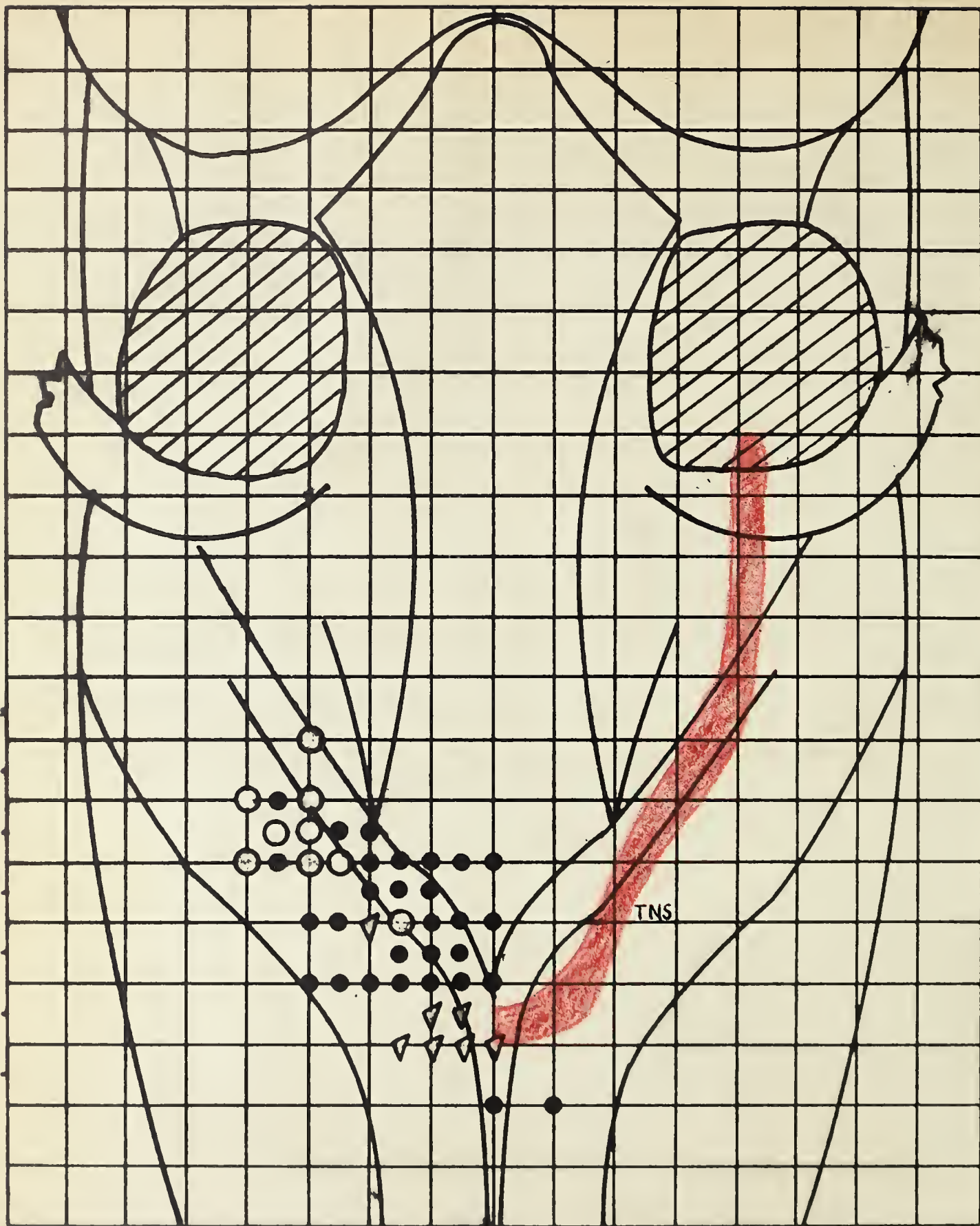
Recording from the rootlets was unsuccessful. The small silver electrodes which had a total recording area of about one mm by 0.2 mm were found to be too large to record from a single rootlet and too apt to be shorted by tissue fluids. The technical problems involved in approaching the rootlets also proved prohibitive.

Retraction of the medulla sometimes had to be employed to make the rootlets visible from above, and the length of nerve thus revealed was only about a millimeter. The accessible portion was situated at the bottom of a bowl-shaped cavity in the bottom of which cerebrospinal fluid and blood readily collected. The dorsal surface of the rootlet bundle was covered with fine, fragile, blood vessels which bled easily. When the rootlets were cut at one end to free them for recording on the pick-up electrodes they contracted to about one-third of their original length.

These problems led to the attempt to record from the rootlets using a micropipette, but this too was unsuccessful. No activity at all was recorded from the surface of the rootlets, and it was found to be impossible to push the fine electrode tip through the connective tissue sheath covering their surface.

This experience has given rise to the opinion that the functional morphology of the vagal rootlets might be best approached using a small steel electrode bent to a hook at the recording end. The rootlets could be studied under oil; the indifferent electrode in this monopolar recording situation being located on the skull of the animal. If this approach was not successful then a possibility exists that a combination of stimulation of parts of the nerve more distally with recording of compound action potentials at the rootlets might produce useful information.

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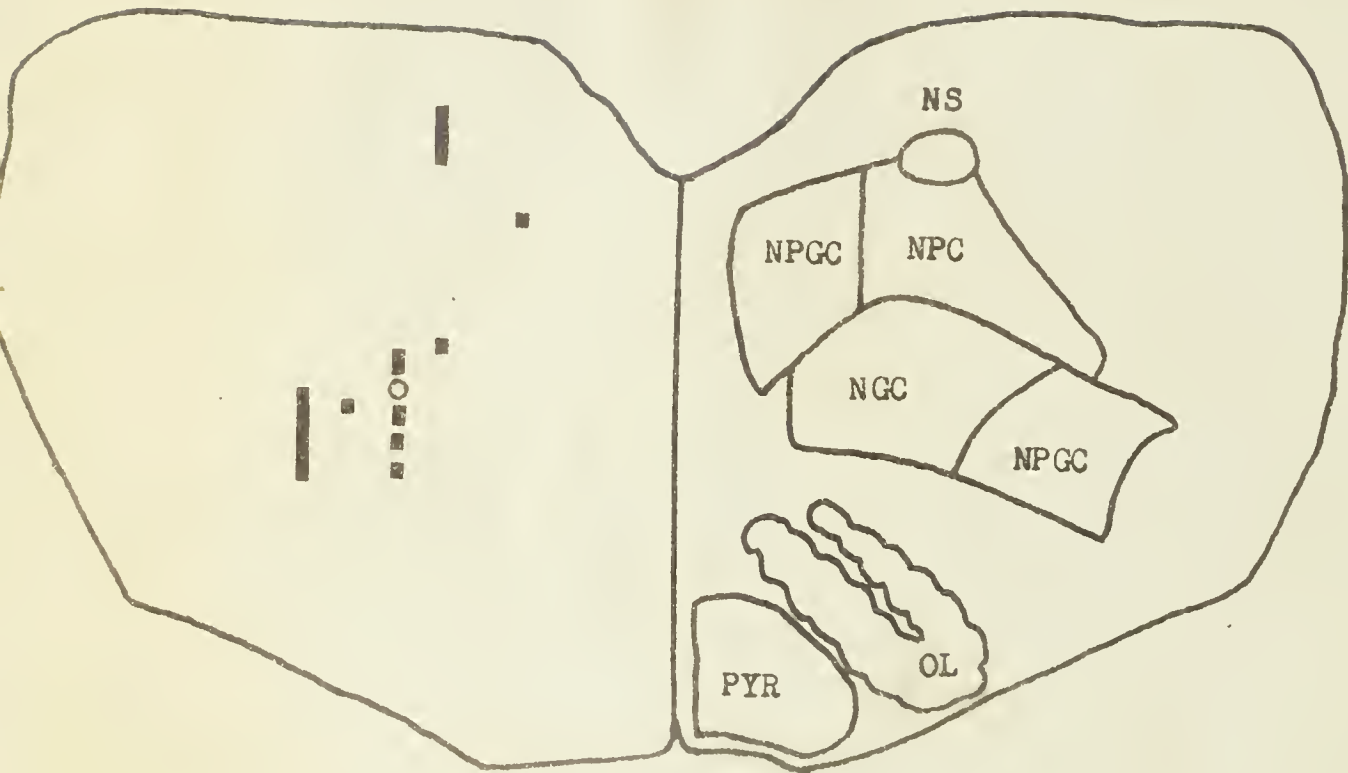
Fig. 15

Diagram of a dorsal view of the medulla of the cat to show the distribution of types of nervous activity recorded.

Large open circles, respiratory activity; triangles, "cardiovascular" types of activity: small filled circles, other types of activity.

The projected location of the tractus and nucleus solitarius (TNS) is shown on the right hand side of the diagram. Letters and the numbers provide reference co-ordinates; the obex is at E9.

A



B

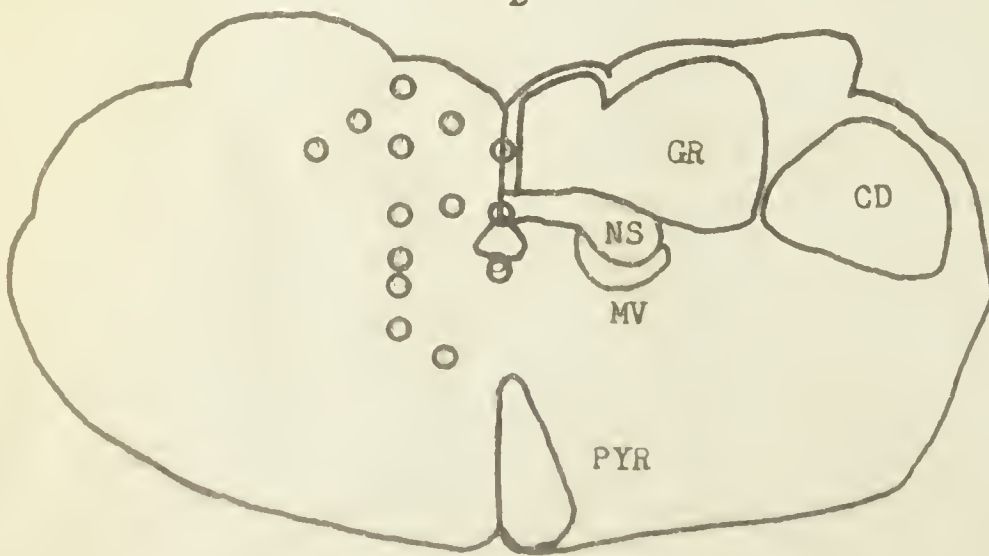


Fig. 16

A. Cross section of the medulla at level I of Fig. 15 showing on the left the positions of recorded respiratory discharges; black, inspiratory discharges; open circle, expiratory discharge.

The right hand side of the diagram shows approximate positions in the cat of the brain stem nuclei described by Olszewski (57) for the human. NS, nucleus solitarius; NPGC, nucleus paragigantocellularis; NGC, nucleus gigantocellularis; NPC, nucleus par^vocellularis; OL, olivary nucleus; PYR, pyramidal tract.

B. Cross section of the medulla at level C of Fig. 15 to show the positions of "cardiovascular" types of discharge recorded. Sites marked by open circles.

Right hand side of section shows estimated positions of CD, caudate nucleus; GR, gracile nucleus; NS, nucleus solitarius; MV, motor nucleus of $\bar{X}$; PYR, pyramidal tract.

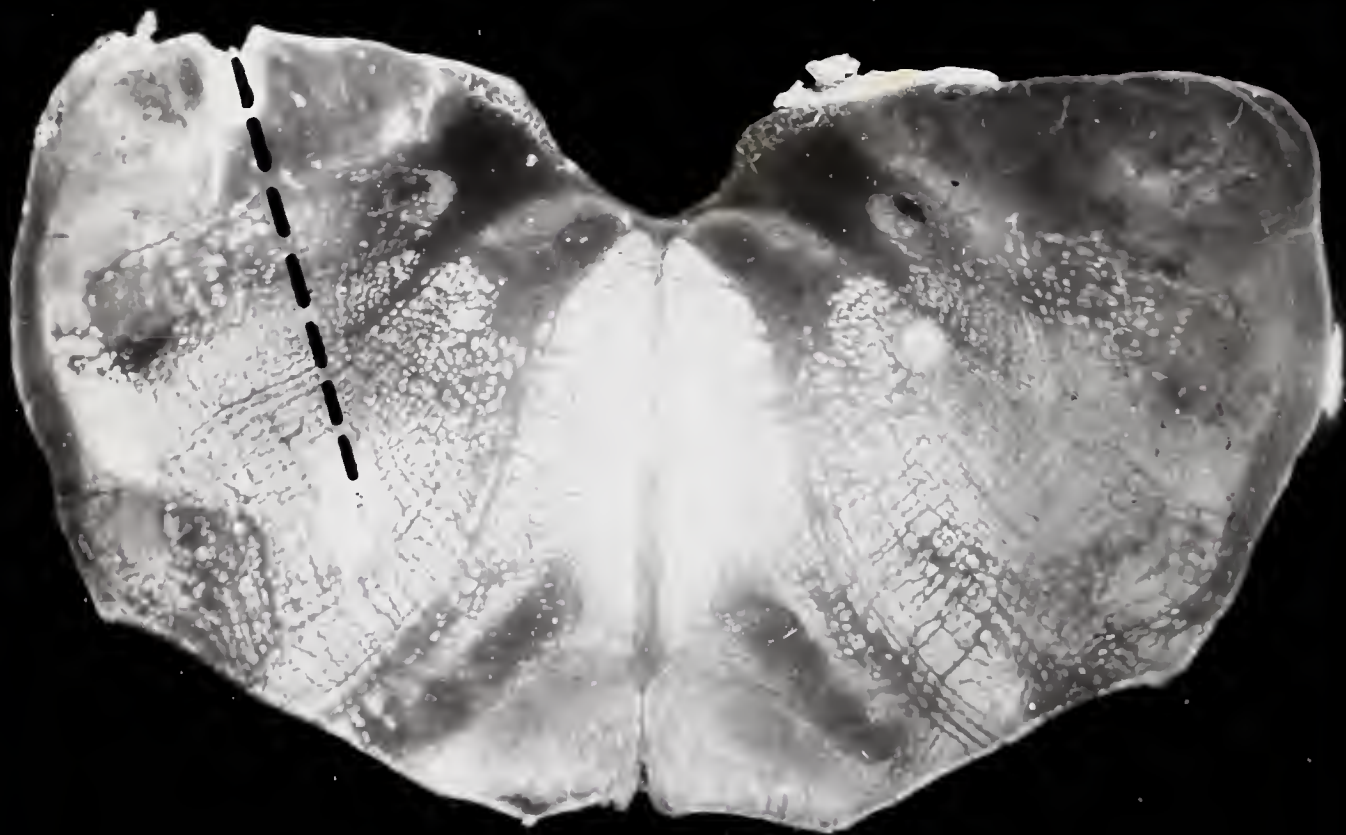
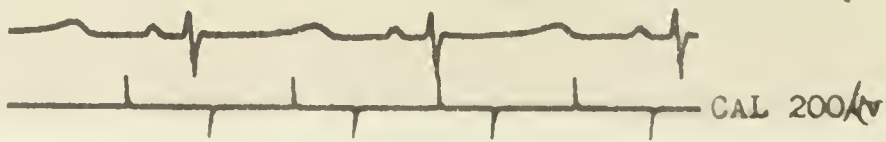
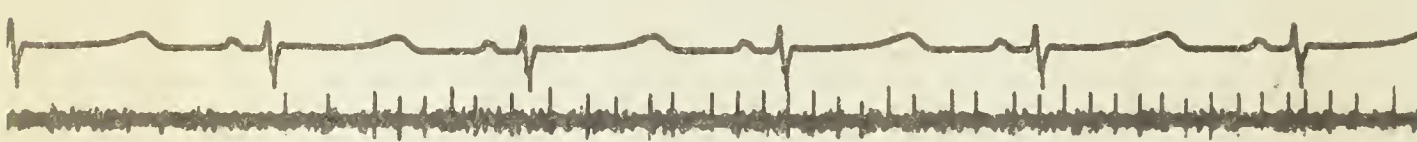


Fig. 17

Photomicrograph of unstained frozen section of medulla at level I of Fig. 15. This figure illustrates the necessity of checking electrode positions histologically. The electrode track, marked by a dotted line, is not vertical because the electrode tip in this case was bent.



CAL 200/v

Fig. 18

Expiratory activity recorded at position J3 of Fig. 15 at a depth of 3.80 m.m. Gaps in the record mark the positions of beginnings of inspirations. Record traces from top downward: ECG, nerve activity with spikes retouched, 60/sec time marker.

(B) The Central Nervous System

The area of the medulla studied in the experiments on the central nervous system is shown in Fig. 15. Forty-five sites were explored, each to a depth of 5 mm. These 45 sites represent separate microelectrode penetrations in 22 cats. The recording position of the electrode tip was checked histologically after the experiment.

Respiratory potentials

Respiratory potentials were recorded at the nine sites shown in Fig. 15. The location of the recorded potentials is also shown in Fig. 16A. The locations are projected onto a plane corresponding with level E of Fig. 16. This plane is shown as a microphotograph in Fig. 17.

The areas from which respiratory potentials were recorded correspond to those described by previous workers (69, 70, 71, 84). Discharges coincident with inspiration (Fig. 13A) were more common than discharges coincident with expiration (Fig. 18). In all cases it was assumed that these potentials were recorded from the cell bodies of the respiratory centers, partly because respiratory potentials were not picked up in the region of primary vagal fibers, and because in one case the electrode track was seen to pass through the posterior tractus; no activity at all was recorded at this level (Fig. 23). Testing the effect of respiratory manoeuvres on respiratory discharges proved to be impossible because the result of sucking or blowing into the trachea was a movement

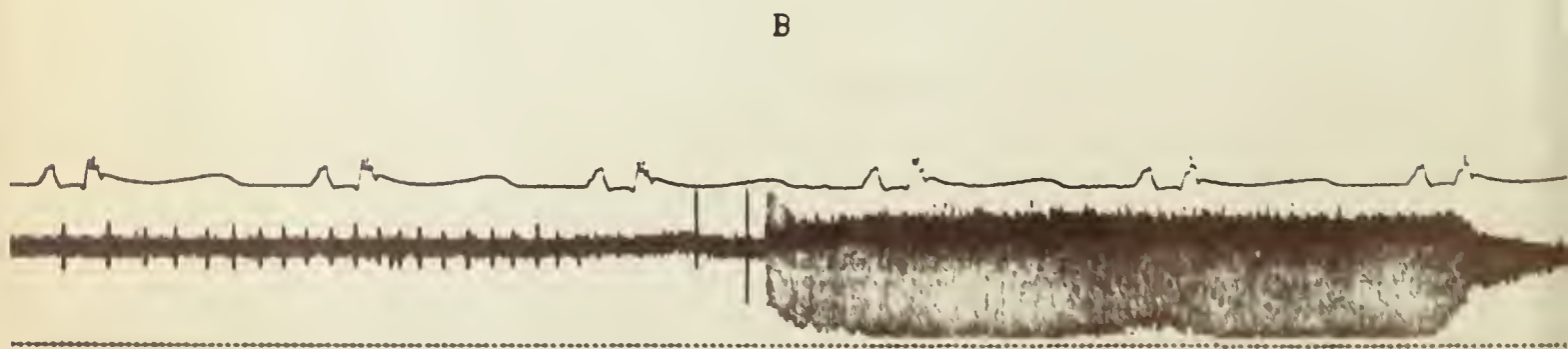
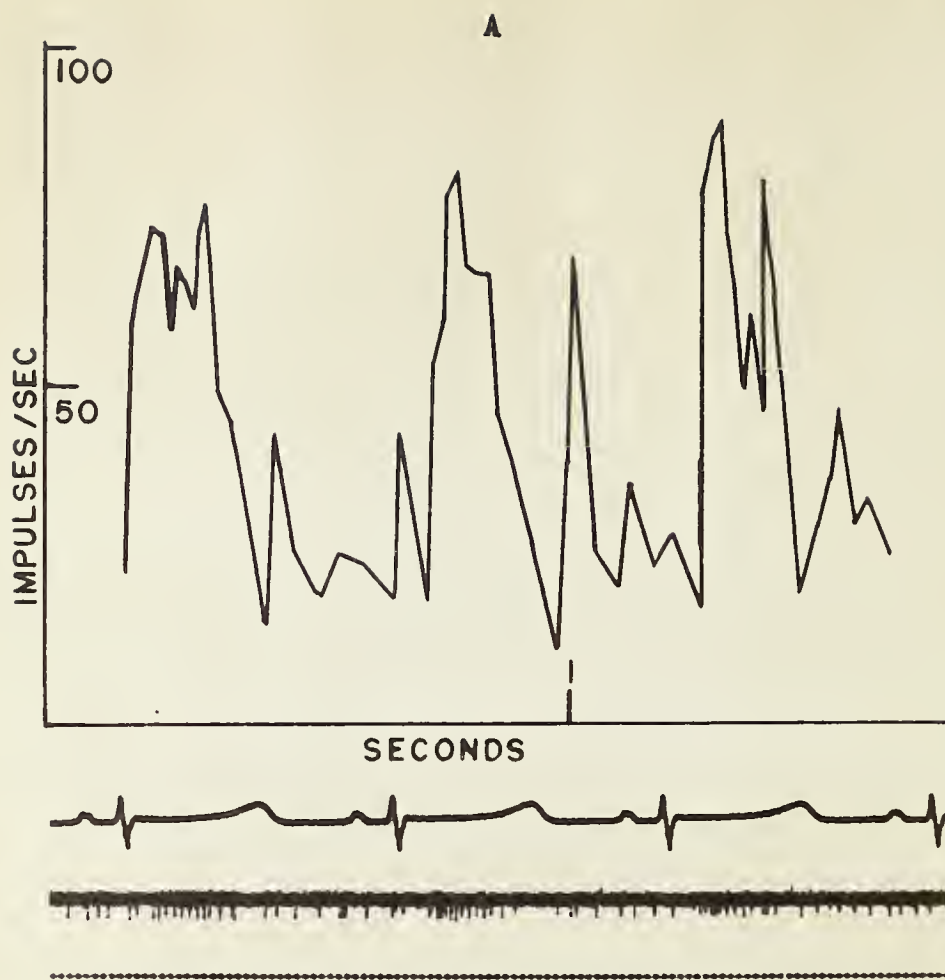


Fig. 19

A. Nerve activity with a cardiac periodicity recorded from position G5 of Fig. 15 at a depth of 0.75 m.m. Frequency plotted uppermost; the record traces from top downward: ECG, nerve activity, 60/sec time marker.

B. Activity from inspiratory center of medulla. The cell responded with an injury discharge to an artificial inflation of the lungs. This manoeuvre caused a movement of the medulla and hence injury of the cell by the electrode.

Traces from top downward: ECG, nerve discharge with spikes retouched, 60/sec time marker.

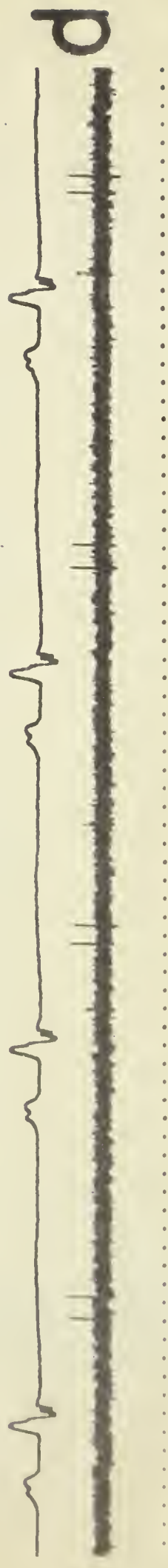
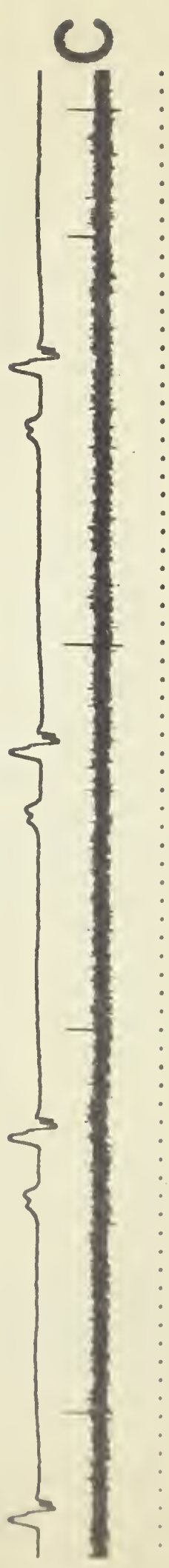
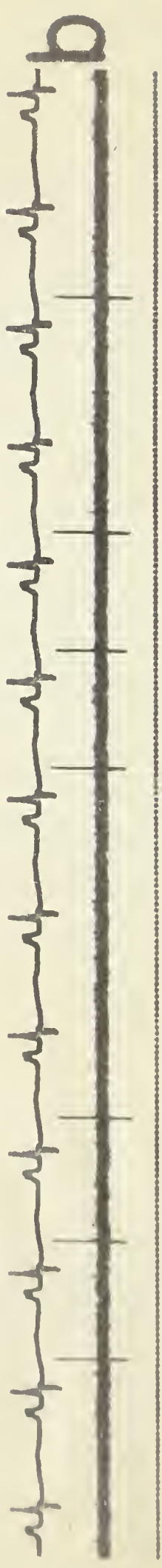


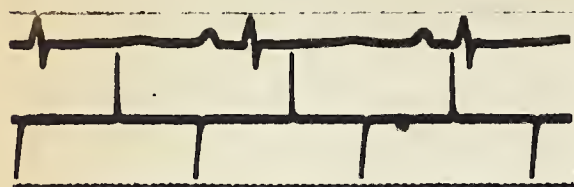
Fig. 20

Types of "cardiovascular" activity recorded from the region of the obex. Positions referred to Fig. 16.

- (a) Position D8 depth 0.5 m.m.
- (b) Position D8 depth 1.75 m.m.
- (c) Position C9 depth 0.5 m.m.
- (d) Position C9 depth 1.8 m.m.

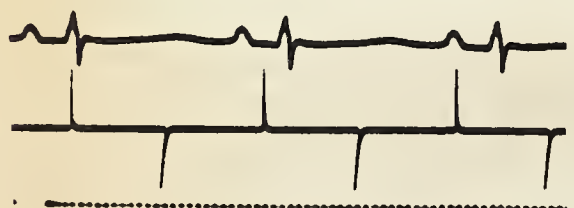
Record traces from top downward: ECG, nerve activity, time marker 60/sec.

A



CAL 500 ∇

B



CAL 1mv

Fig. 21

A. Continuous discharge recorded at position C7
of Fig. 15 at depth 1.75 m.m.

B. Random discharge pattern recorded at position G5
of Fig. 15 at depth 3.50 m.m.

Record traces from top downward: ECG, nerve activity
with spikes retouched, 60/sec time marker.

of the brain stem and, usually, injury of the cell under observation (Fig. 19B).

Cardiovascular rhythms

Cells firing with some type of cardiovascular rhythm were recorded in areas shown in Figs. 15 and 16B. The types of discharges recorded are shown in Figs. 19A and 20. This type of discharge was often recorded in areas just around the obex; the medulla here is very vascular as is shown in Fig. 25. Unfortunately this area is also one in which movement artefacts are recorded, possibly due to the vascularity of the brain and its less rigid structure at the beginning of the spinal canal.

Continuously firing cells

At many places in the recording area continuously firing cells were observed. The discharge from one such cell is shown in Fig. 21A. These cells were often found near the areas of respiratory discharge, but not invariably so. The significance of these and other patterns is discussed later.

Randomly firing cells

Random patterns of discharge were picked up at all recording sites. A short sequence of such a discharge is shown in Fig. 21B.

Other records

Many records were obtained of patterns of real nerve discharge, the rhythms of frequency or amplitude of which were believed to be artefactual. Some such patterns are shown in

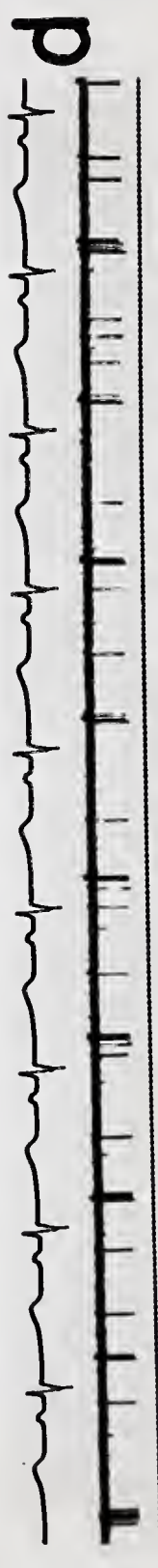
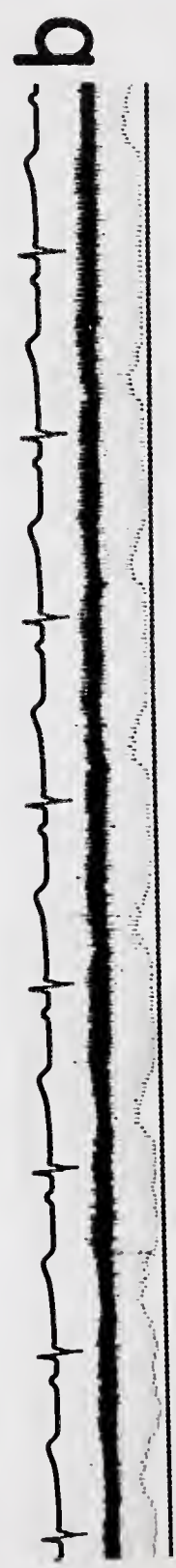
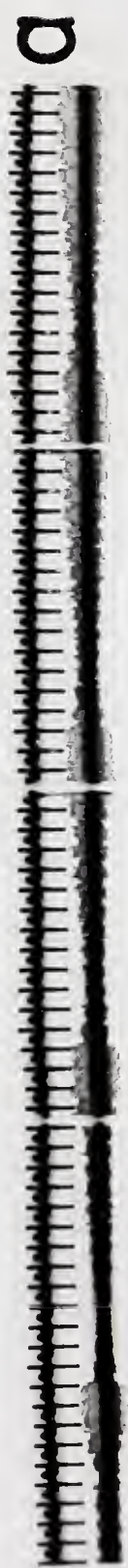


Fig. 22

Various types of artefactual nerve discharge recorded from the medulla.

Record traces from top downward: ECG, nerve activity, 60/sec time marker.

The gaps in trace (a) represent the beginnings of the inspiratory phase of the respiratory cycle. Explanation of the records in text.

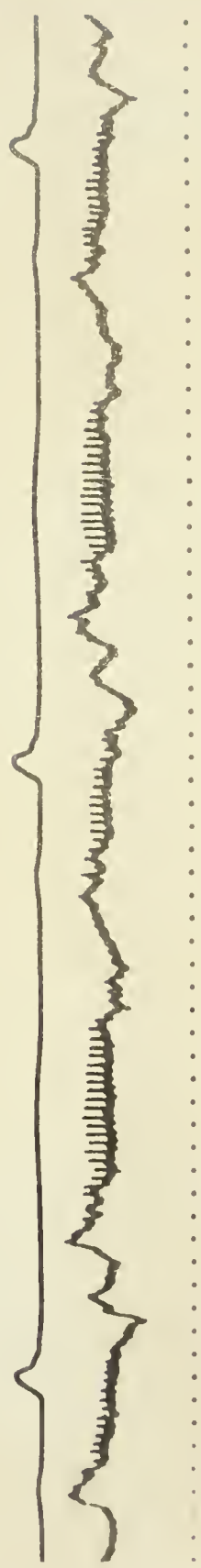


Fig. 23

Record showing a cardiovascular type of artefact. The nerve is firing at a constant frequency but is recorded only during suitable shifts of the medulla caused by the vascular pressure pulse.

Record traces from top downward: ECG, nerve activity with spikes retouched, 60/sec time marker.

Figs. 22 and 23. The general types observed were: action potentials whose height was varied as the cell moved toward and away from the electrode, cells whose rate of firing was influenced by the electrode, and recordings made intermittently from cells firing at a steady rate.

Fig. 22 traces (c) and (d) show a cell possibly firing in response to movement of the medulla caused by the vascular pressure pulse. Trace (d) could represent a cell which was firing at some slow rate naturally, but is being speeded up by the irritation of movement. Traces (a) and (b) show a continuously firing neurone with variations in spike height caused by both respiratory and cardiac movements. Fig. 23 shows clearly the movement of the medulla caused by the vascular pressure pulse. The nerve impulses in this figure represent a nerve firing at constant frequency; the pulses show above the base line only during suitable shifts of the medulla.

The significance of the patterns described above, both actual and artefactual, is taken up later in the discussion.

DISCUSSION

The distribution of recordings of known types of respiratory discharges in the central nervous system (Fig. 16) suggests that the spike potentials recorded extracellularly were from cell bodies rather than from axons. Some support to this contention has been given in a recent paper by Freygang and Frank (87) in which they conclude that extracellular potentials recorded from single spinal motoneurons originated in the cell bodies. This idea is further borne out by the experiments described previously on the peripheral nervous system; spike potentials were easily recorded from the nodose ganglion while few recordings were obtained in the vagal nerve trunk. Respiratory patterns were obtained about 1 cm. distal to the nodose ganglion, but whether these were recorded from a cell body or from a transfixed axon is uncertain. It is known (85) that if axons are approached or transfixed by microelectrodes with ultrafine tips ($0.5\ \mu$ external diameter), then spike potentials can be recorded from myelinated axons.

Some insight into the failure to record spike potentials extracellularly from axons was given by the experiment in which attempts were made to record from the aortic depressor nerve. In this experiment a potential change in the complete nerve was recorded when the aortic depressor volley passed up the nerve in each cardiac cycle. This was the result obtained when the nerve was surrounded by its connective tissue sheath. Yet, when the

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Received of the Hon. Secy. of the Interior

(L. I.) \$100.00 for the purchase of land

for the purpose of the National Forest

in the State of California.

The sum of \$100.00 is hereby acknowledged

as being the full amount of the purchase

price of the land.

Witness my hand and the seal of the

Department of the Interior at Washington

D. C. this 1st day of January 1911.

Very truly yours,

John D. Brown, Secretary of the Interior

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nerve sheath was split open in saline no potential charge was recorded. A possible explanation for this phenomenon is that in the first case the connective tissue sheath, being a medium of high specific resistivity, caused the recording situation to approximate that in which a nerve is studied while immersed in oil. It is known (72) that in this case the recorded potential is caused by a current travelling longitudinally to the nerve and that the spike "seen" by the electrode is monophasic. However, if a nerve is resting in a volume conducting medium, i.e. in saline or in the central nervous system, then the spike potential recorded extracellularly is an expression of the radial current caused by depolarization. In this case the electrode "sees" a triphasic spike caused by a source of current followed by a sink of current, followed by a source of current. The currents from a single axon during a single depolarization, if integrated through time, sum to zero. If this is the case, then a number of depolarization cycles out of phase with each other by varying amounts and superimposed will present zero current to the electrode at any point in time. Hence no spike potentials will be recorded. The superimposition of a number of depolarization cycles in this way was the likely situation existing in the peripheral aortic depressor nerve. Presentation of radially directed currents to the electrode may then have been caused by splitting the connective tissue sheath under saline.

The same argument can be directly applied to the

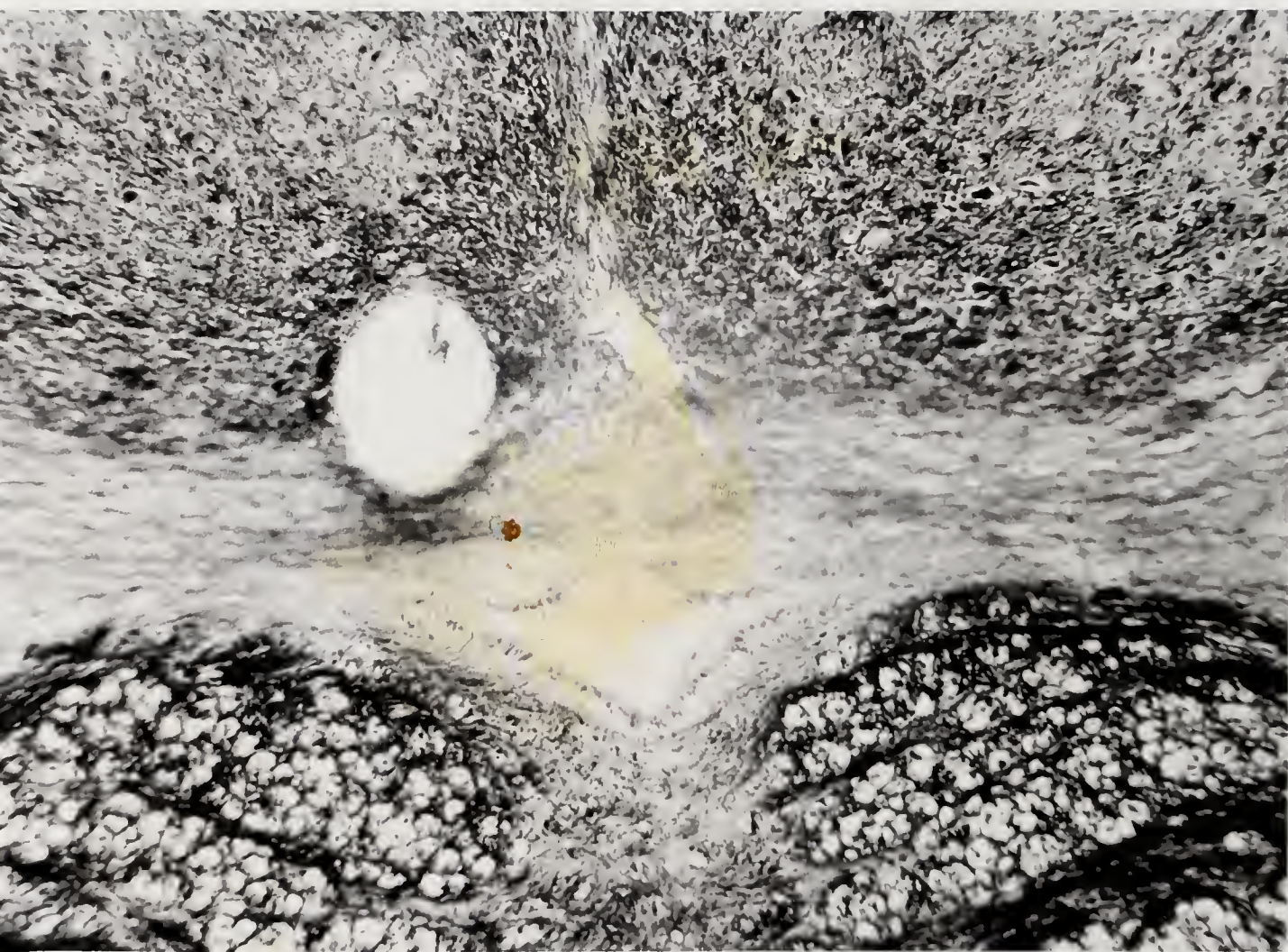


Fig. 24

Photomicrograph of the commissure of Cajal containing the fibers of the tractus solitarius which pass over the spinal canal at this point. The oral hole was left by the shank of an electrode which passed obliquely through the plane of section. The electrode hole cuts through the dorsal part of the commissure. No nervous activity was recorded at this level.

Weil's stain.

situation in the central nervous system. Here the vagus nerve runs in a tight bundle, the tractus solitarius, in a volume conducting medium. The hypothesis is presented that in this situation extracellular, that is, extra-axonal, electrodes will not record spike potentials. Some support was given to this idea when evidence was obtained that an electrode had passed through the tractus solitarius at the commissure of Cajal (Fig. 24); no spike potentials were recorded at this depth. Impulses were recorded using the same needle advanced further into the medulla.

It is useful to summarize the factors which influence the recording of spike potentials with a microelectrode.

1. The shape of the spike depends on the direction of the current at the recording tip of the electrode (77).

2. The size of the spike is influenced by several factors; these will be called attenuating factors.

- a. The size of the actual sink or source of current is reflected in the recorded spike height.

- b. The attenuation of the spike height is a function of the distance of the electrode tip from the original sink or source. Attenuation is approximately proportional to the cube of this distance.

- c. Mixing of currents from separate sources can cause attenuation, sometimes reinforcement, of the spike height.

- d. The electrode tip diameter will influence the

spike size. A larger tip diameter will average the potentials over a greater area, and hence cause greater attenuation.

e. The noise introduced into the electrical circuitry by the electrode will put a practical limit to the volume over which a depolarization can be recorded. That is, when the other attenuating factors reduce the recorded spike height to the amplitude of the electrode noise the limit of resolution of the electrode has been reached.

The noise level of the electrode is given by

$$RT^E_{RMS} = 0.13 \sqrt{R(f_2 - f_1)}$$

Where, RT^E_{RMS} is the root mean square noise voltage at room temperature in μv .

R is the resistance of the electrode in

$f_2 - f_1$ is the recording bandwidth in cycles/sec.

(Equation from the Grass Instrument Co. manual on the P5 amplifier).

The graph of Fig. 25 shows the root mean square noise voltage and the "peak to peak" noise voltage calculated for an electrode in the range of resistances used in this series of

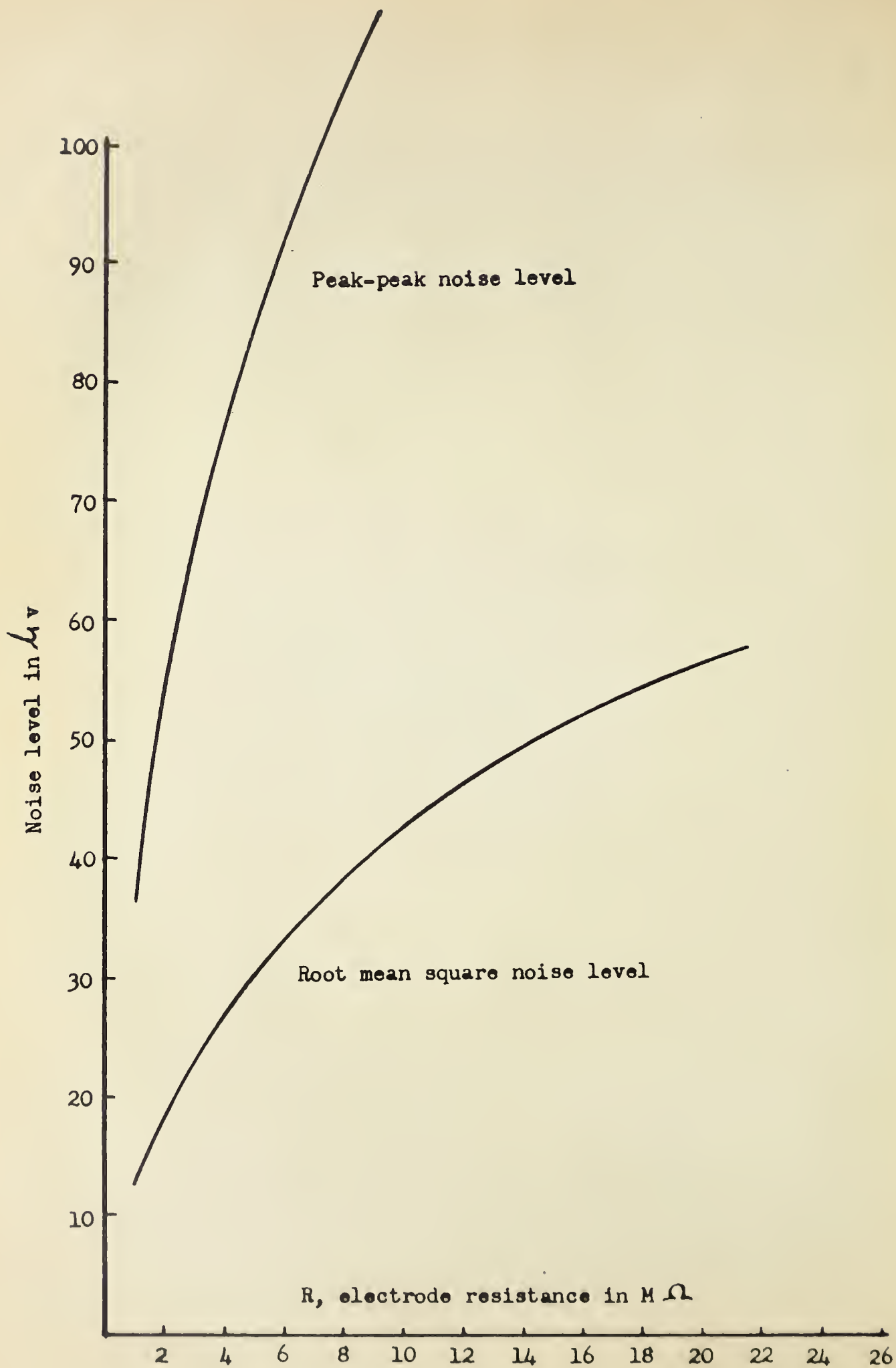


Fig. 25

Resistance-noise level diagram for electrodes recording over a bandwidth of 10 kilocycles.

experiments.

Effects 2c and 2d account for the failure in these experiments to record extracellularly from the tractus solitarius. Effect 2e offers some contradiction to the objection to extracellular recording that one is not sure where the recorded potentials originate because noise will set a boundary through which distant potentials cannot be recognized.

It can be said then that this investigation has contributed to the understanding of the type of recording that is possible to obtain with a microelectrode extracellularly. It is concluded that the records obtained from the central nervous system represented the activity of the cell soma, in agreement with the opinion that Woldring formed while studying the respiratory centers (84) and with the recent observations of Freygang and Frank on spinal motoneurons.

Effect 2a, that the size of the source or sink of current will affect the height, and hence the likelihood of recording, the spike potential, combines with the observation of Cajal (12) on the small size of cells in the nucleus solitarius and nucleus commissuralis to suggest that it will be difficult, and perhaps impossible, to record from such cells with electrodes of the tip diameters used (2-20 μ). It is very likely that small cells could be approached better with intracellular techniques using hyperfine electrodes.

The idea that the size of the sink or source of current influences the size of the spike potential recorded can be developed further. If a cell may be regarded as being a sphere having a membrane which passes an amount of current Q per unit surface area on depolarization, then the total current generated by the cell will be $Q 2/3\pi R^2$, where R is the radius of the sphere. A spherical cell, then, causes at a point outside it a current density proportional to the square of its radius. Allowing that cells are not spheres, it can still be said that the ratio of the currents developed by two cells of different sizes, but with similar surface membranes, will be approximately equal to the ratio of the squares of some similar linear dimension of each cell. This effect will cause a large cell to be easier to record from, both because the probability of coming within its volume of influence will be greater, and because the potentials recorded when close to the cell will be much larger than those recorded from a small cell.

From experience in this series of experiments and from the literature, it would seem likely that respiratory potentials, from the ease with which they can be recorded, and considering their large size, would be originating in large cells. As these cells have a motor function they might be expected to show the type of morphology associated with motor neurones. Fig. 16 shows that respiratory potentials were often recorded from an area of

the medulla which corresponds closely with that of the groups of cells which Olszewski calls the nucleus gigantocellularis and nucleus paragigantocellularis. The cells he describes are of the morphological motor type. It is suggested that the respiratory potentials observed were originating in the cells of the nucleus gigantocellularis, and perhaps in the nucleus paragigantocellularis. It has, so far, been concluded that both the size of the electrode and the size of the cell have a marked effect on the possibility of recording spike potentials from the cell. With this limitation in mind, that it is possible that representatives of all the patterns of cell activity within the recording area were not obtained, the significance of the recorded potentials can be discussed.

The various types of patterns of cell activity recorded in the medulla are described in the Results. It is possible that any of these types is associated with central visceral nervous activity. Those most likely to have originated from the nucleus solitarius are the respiratory potentials recorded in the most dorsal region shown in Fig. 16 and the cardiovascular type discharges recorded from the region of the obex. Most interest in this investigation centered on volleys having a relationship to the cardiac cycle. Because the resemblance between the central inspiratory volleys and those respiratory volleys travelling to the medulla in the vagus nerve was so striking, it was initially thought that central activity of a type resembling the afferent

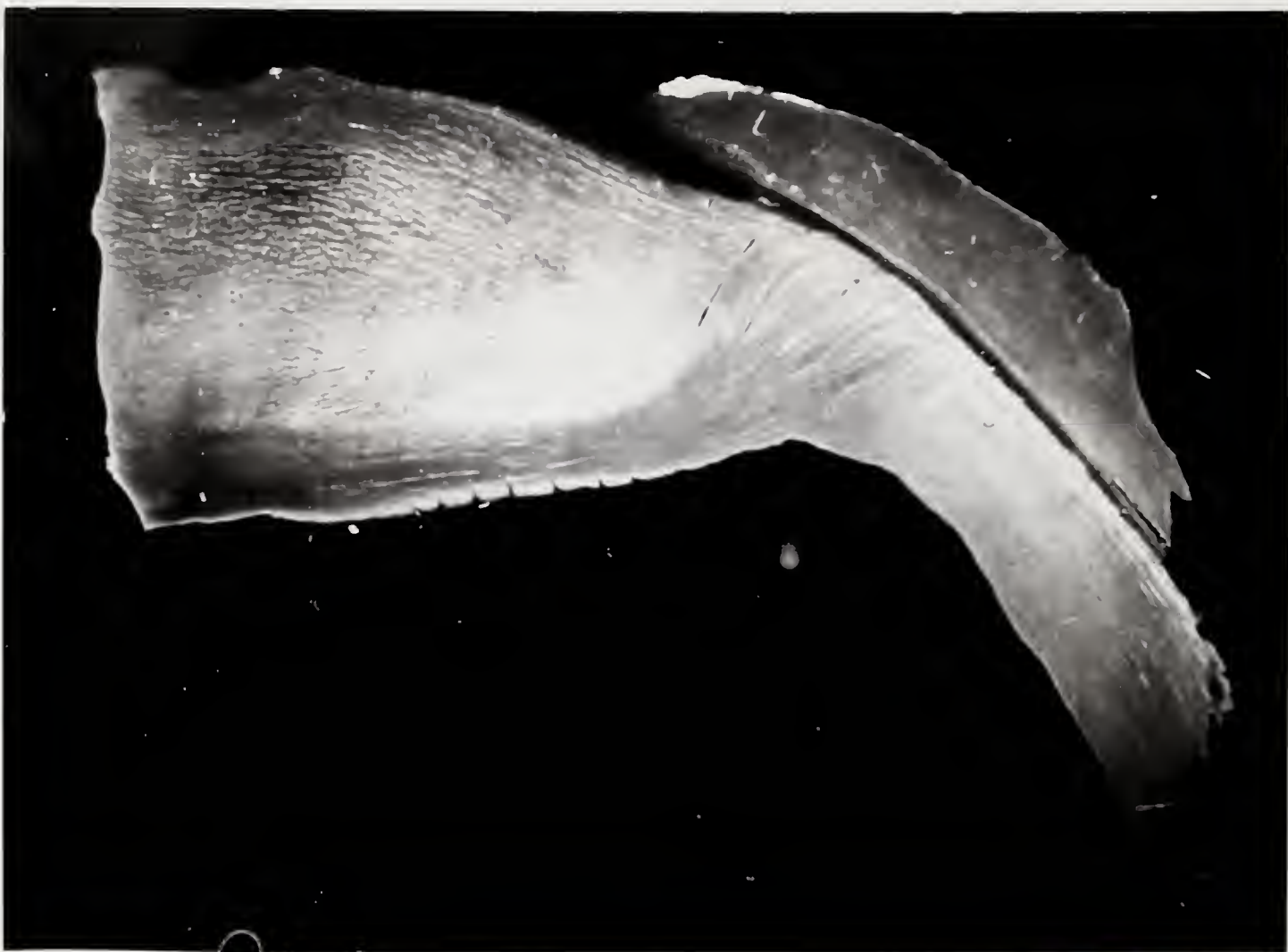


Fig. 26

Photomicrograph of a sagittal frozen section of the medulla showing the blood vessels entering ventrally. A large blood vessel can also be seen in the dorsal medulla just below the obex.

cardiac volleys would be recorded. However, none of this type was observed. This is consistent with the contention that the activity in fiber tracts was not recorded in the central nervous system, but it still leaves in doubt the nature of the discharge of the cells at the first synapse of the nerve fibers concerned with cardiac activity. The type of activity related to the cardiac cycle that has been recorded has been shown in the illustrations under Results.

Nerve cells are sensitive to physical distortion and some evidence exists to suggest that the cardiac timed volleys recorded may have been artefacts. The evidence for a strong tendency toward the formation of artefacts in these experiments has already been commented on in the Results. Fig. 26 shows that the region from which these recordings were made is very vascular; it is hence liable to pulsatile movement.

In the future, microelectrode recording from the brain stem should be combined with some form of precaution which would eliminate movement artefacts. It is planned to eliminate movements caused by respiration by opening the thorax wall and respiring the cat with a pump. Mechanical fixation of the medulla may enable the movements due to the vascular pulse to be dampened.

Although such precautions are commonly used for intracellular recording, it was decided during the planning of these experiments to avoid opening the chest for two reasons. The exploratory nature of the study, using rather large microelectrodes

extracellularly was not thought to require such a high degree of freedom from artefacts as it was believed that central activity would be prominent and easy to distinguish from artefactual activity. It was also considered necessary to avoid any operative procedure which might reduce the efficiency of cardiac filling and thus decrease the amount of activity in cardiac receptors, particularly those in the atria.

Representative patterns of other types of cell activity were obtained from the brain stem. It is possible that some of these represented central visceral nervous activity, although this would have passed unrecognized owing to the present lack of knowledge of the ways in which information is carried across a synapse in this region. Assuming that the central recordings were made from cells which were at least second order neurons, then two possible situations hold.

1. Cardiac nervous activity was not recorded due to limitations of technique.
2. Cardiac nervous activity was recorded but it was not of a type which is a simple trans-synaptic image of the primary afferent volley.

If the second case holds then the route by which the information in the primary afferent volley is transmitted to higher brain centers could be of two general types:

1. A simple direct route in which information of the single functional modality is carried either with no loss

of information, an ideal situation, or with some loss of information, a more probable occurrence.

2. A route in which information from several functional modalities is integrated. This type of interneurone has been observed by Skoglund in the spinal cord (75).

It is conceivable that information carried in an integrated form could be "unscrambled" at a receiving center, but this would not alter the fact that recordings obtained from the channel carrying integrated signals would be of a complex form. If no "unscrambling" center existed and the information were integrated in some sector of a reflex arc, then it would be expected that the reflex activity would be influenced by more than one type of stimulus, that is, by all those modalities integrated.

Assuming that the afferent cardiac volley is not transmitted to higher brain centers as a simple trans-synaptic image, then several ways exist in which information concerning the afferent volley alone could be transmitted. These several ways all depend on the basic effect of one neurone on another, a modification of neurone excitability by excitation or inhibition. These effects have been described in detail by Eccles (22). The modification of excitability can be either above or below the threshold value needed to produce a spike potential. The changes of state of the second neurone could be:

1. Modification of a non-firing state into either a firing state or another, different, non-firing state.

2. Modification of a constantly firing state (c.f. spinal interneurons described by Skoglund (75)).

3. Modification of a periodically firing state.

If the second order neurone were an integrative point then the output pattern of spike potentials by this neurone could be expected to be correspondingly more complex.

During the writing of this discussion an important paper by Bonvallet and Sigg (9) has come to light. This paper describes experiments in which single fiber activity was recorded from the vagal rootlets of the cat. The technique used was identical to that which has already been suggested under the Results, namely, the use of a fine single steel hook immersed in oil, the indifferent electrode being attached to the animal's skull.

The results of the work done by these authors shows that there is no simple separation of fibers in the vagal rootlets into functional modalities. Thoracic and abdominal receptors are mixed in any given rootlet. However, evidence is presented that a greater number of cardiovascular fibers are present in the anterior rootlets than in the posterior ones. The opposite situation holds for pulmonary nerve fibers which seem to be contained in greater numbers in the posterior rootlets. Both dorsal and ventral rootlets contain some sensory fibers but the majority of the sensory fibers are found in the dorsal rootlets; this finding is in accordance with the histological findings of Foley and DuBois (24).

In view of these findings it seems that future approaches to the problem of the nervous control of the cardiovascular system will not be a simple matter of stimulating a large number of, for example, atrial fibers in one of the vagal rootlets. The basic methods of central nervous investigation are still generally applicable: stimulation, ablation, and recording. Recording techniques could be applied in two ways: first, the behavior of cells in the brain stem can be studied using either intra or extracellular recording techniques, second, the patterns of output activity from the vasomotor centers can be studied using single fiber recording techniques in the peripheral vagus nerve. Both these techniques could be combined with suitable cardiovascular manoeuvres which would enable the activity to be recognized as cardio or vasomotor. The evidence obtained in this way on the nature of the ultimate output from the brain stem centers could then be applied to differentiate in central recordings this type of activity from interneurone activity which would also depend in some way on the cardiovascular situation. As yet it seems premature to attempt to carry the investigation to brain levels higher than the brain stem.

CONCLUSIONS

1. An exploration of the region of the tractus solitarius was carried out using microelectrodes ranging in size from 2-20 μ . With the techniques used in this investigation no recording was made that unequivocally represented activity from primary afferent cardiovascular neurones.

2. Inspiratory activity was recorded from the region of the tractus solitarius but no evidence exists that this activity did not originate in the inspiratory center.

3. The adequacy of the microelectrode technique was demonstrated in the readiness with which respiratory activity was recorded in the centers already described by Woldring (84).

4. Several types of artefact liable to be met with in the use of microelectrodes have been described.

5. Other types of central nervous discharge were recorded but not interpreted. Among these were discharges with a cardiovascular rhythm which was not shown to be an artefact.

6. Single fiber potentials were recorded from the nodose ganglion using microelectrodes.

7. Attempts to record from the vagal rootlets using miniature paired pickup electrodes and micropipettes were unsuccessful.

8. The limits of the microelectrode technique were theoretically considered.

BIBLIOGRAPHY

1. Adrian, E. D. (1933). Afferent impulses in the vagus nerve and their effect on respiration. *J. Physiol.*, 79:332.
2. Agostini, E., Chinnock, J. E., Daly, M. De B., and Murray, J. G. (1957). Functional and histological studies of the vagus nerve and its branches to the heart, lungs, and abdominal viscera in the cat. *J. Physiol.*, 135:182.
3. Allen, W. F. (1932). Formatio reticularis and reticulo spinal tracts, their visceral functions and possible relationships to tonicity and clonic contractions. *J. Wash. Acad. Sc.*, 22:490.
4. Anderson, F. D., and Berry, C. M. (1956). An oscillographic study of the central pathways of the vagus nerve in the cat. *J. Comp. Neurol.*, 106:163.
5. Aviado, D. M., Li, T. H., Werner, K., Schmidt, C. F., Turnbull, G. L., Peskin, G. W., Hess, M. E., and Weiss, A. J. (1957). Respiratory and circulatory reflexes from the perfused heart and pulmonary circulation of the dog. *Amer. J. Physiol.*, 165:261.
6. Aviado, D. M. and Schmidt, C. F. (1955). Reflexes from stretch receptors in blood vessels, heart and lungs. *Physiol. Rev.*, 35:247.
7. Bach, L. M. N. (1948). The role of bulbar facilitatory and inhibitory systems in vasomotor and respiratory systems. *Fed. Proc.*, 7:4.
8. Bach, L. M. N. (1952). Relationships between bulbar respiratory, vasomotor, and somatic facilitatory and inhibitory centers. *Amer. J. Physiol.*, 171:417.
9. Bonvallet, M. and Sigg, B. (1958). Etude electrophysiologique des afferences vagales au niveau de leur penetration dans le bulbe. *J. Physiologie*, 50:63.
10. Boss, J. and Green, J. H. (1956). The histology of the common carotid baroreceptor areas of the cat. *Circ. Research*, 4:12.

11. Brodal, A., Szabo, Th. and Torvik, A. (1956). Cortico-fugal fibres to sensory trigeminal nuclei and nucleus of solitary tract. An experimental study in the cat. *J. Comp. Neurol.*, 106:527.
12. Cajal, S. R. (1907). *Histologie du systeme nerveux*. Tome 1, Paris: Maloine.
13. Chapman, K. M. (1959). Proceedings of the western regional group division of medical research, National Research Council of Canada.
14. Coleridge, J. C. G., Hemingway, A., Holmes, R. L., and Linden, R. J. (1957). The location of atrial receptors in the dog; a physiological and histological study.
15. Constantin, Le Roy, L. (1959). Effect of pulmonary congestion on vagal afferent activity. *Amer. J. Physiol.*, 196:49.
16. Daly, M. De B., and Evans, D. H. L. (1953). Functional and histological changes in the vagus nerve of the cat after degenerative section at various levels. *J. Physiol.*, 120:579.
17. Donaldson, P. E. K. (1958). *Electronic apparatus for biological research*. London: Butterworth.
18. Domino, E. F. A pharmacologic analysis of some reticular and spinal cord systems. (1958). Henry Ford Hospital International Symposium: Reticular Formation of the Brain. Boston, Little, Brown and Company.
19. Douglas, W. W. and Schaumann, W. (1956). A study of the depressor and pressor components of the cat's carotid sinus and aortic nerves using electrical stimuli of different intensities and frequencies. *J. Physiol.*, 132:173.
20. Du Bois, F. and Foley, J. (1936). Experimental studies on the vagus and spinal accessory nerves in the cat. *Anat. Rec.*, 64:285.
21. Eod, H.W., Green, J. H., and Neil, E. (1952). A comparison of the effects of pulsatile and non-pulsatile blood flow through the carotid sinus on the reflexogenic activity of the sinus baroreceptors in the cat. *J. Physiol.*, 118:509.
22. Eccles, J. C. (1957). *The Physiology of the Nerve Cells*. Baltimore: The Johns Hopkins Press.

23. Elftman, A. G. (1943). The afferent and parasympathetic innervation of the lungs and trachea of the dog. *Amer. J. Anat.*, 72:1.
24. Foley, J. and Du Bois, F. (1934). An experimental study of the rootlets of the vagus nerve in the cat. *J. Comp. Neurol.*, 60:137.
25. Gauer, O. H., Henry, J. P., Sieker, H. O., and Wendt, W.I., (1954). The effect of negative pressure breathing on urine flow. *J. Clin. Invest.*, 33:287.
26. Gauer, O. H., Henry, J. P., and Sieker, H. O. (1956). Changes in central nervous pressure after moderate hemorrhage and transfusion in man. *Circ. Research*, 4:79.
27. Green, J. H. (1953). A new baroreceptor area in the cat. *J. Physiol.*, 122:70P.
28. Green, J. H. (1954). Further baroreceptor areas associated with the right common carotid artery in the cat. *J. Physiol.*, 123:41P.
29. Head, H. (1889). On the regulation of respiration. *J. Physiol.*, 10:1.
30. Henry, J. P., Gauer, O. H. and Reeves, J. C. (1956). Evidence of the atrial location of receptors influencing urine flow. *Circ. Research*, 4:85.
31. Henry, J. P., Gauer, O. H. and Sieker, H. O. (1956b). Effect of moderate changes in blood volume on left and right atrial pressures. *Circ. Research*, 4:91.
32. Henry, J. P., and Pearce, J. W. (1956). The possible role of cardiac atrial stretch receptors in the induction of changes in urine flow. *J. Physiol.*, 131:572.
33. Herrick, C. J. (1944). The fasciculus solitarius and its connections in amphibians and fishes. *J. Comp. Neurol.*, 81:307.
34. Heymans, C., and Neil, E. (1958). Reflexogenic areas of the cardiovascular system. London: J. & A. Churchill, Ltd.
35. Hirsch, E. F., and Orme, J. F. (1947). Sensory nerves of the human heart. *Arch. Path.*, 44:325.

36. Howe, A. (1956). The vasculature of the aortic bodies in the cat. *J. Physiol.*, 134:311.
37. Howe, A. and Diamond, J. (1956). Chemoreceptor activity in the aortic bodies of the cat. *J. Physiol.*, 134:319.
38. Hubbard, S. J. (1958). A study of rapid mechanical events in a mechanoreceptor. *J. Physiol.*, 141:198.
39. Hubel, D. H. (1957). A tungsten microelectrode for recording from single units. *WRAIR-42-57*.
40. Iggo, A. (1955). Tension receptors in the stomach and the urinary bladder. *J. Physiol.*, 128:593.
41. Iggo, A. (1956). Central nervous control of gastric movements in sheep and goats. *J. Physiol.*, 131:248.
42. Iggo, A. (1957). Gastric pH receptors with slowly conducting centripetal fibres. *J. Physiol.*, 137:17P.
43. Iggo, A. (1957). Gastro-intestinal tension receptors with unmyelinated afferent fibres in the vagus of the cat. *Q. J. Exp. Physiol.*, 42:130.
44. Jones, R. L. (1937). Cell fibre ratios in the vagus nerve. *J. Comp. Neurol.*, 67:469.
45. Kappers, C. V. A., Huber, G. C. and Crosby, E. C. (1936). The comparative anatomy of the nervous system of vertebrates, including man. Vol.1. New York: Macmillan & Company.
46. Landgren, S. (1952). On the excitation mechanism of the carotid baroreceptors. *Acta Physiol. Scand.*, 26:1.
47. Larsall, O. (1921). Nerve terminations in the lung of the rabbit. *J. Comp. Neurol.*, 33:105.
48. Larsall, O. and Mason, M. L. (1921). Experimental degeneration of the vagus nerve and its relation to the nerve terminations in the lung of the rabbit. *J. Comp. Neurol.*, 33:509.
49. Lim, R. K. S., Wang, S. C. and Li, C. L. (1938). On the question of a myelencephalic sympathetic center VII. The depressor center of a sympathoinhibitory center. *Chinese. J. Physiol.*, 13:61.

50. Lumsden, T. (1923). Observations on the respiratory centers in the cat. *J. Physiol.*, 57:153.
51. Magoun, H. W. and Rhines, R. (1946). An inhibitory mechanism in the bulbar reticular formation. *J. Neurophysiol.*, 9:165.
52. McEachern, C. G., Manning, C. W., and Hall, G. E. (1940). Sudden occlusion of coronary arteries following removal of cardio-sensory pathways. *Arch. Int. Med.*, 65:661.
53. Mannier, M. M. (1938). Physiologie des formations reticulees. II Respiration: Effets de l'excitation faradique du bulbe chez le chat. *Rev. Neurol.*, 69:517.
54. Montgomery, A. V. and Holmes, J. H. (1955). Gastric inhibition of the drinking response. *Amer. J. Physiol.*, 182:227.
55. Nonidez, J. F. (1937). Identification of the receptor areas in the venae cavae and pulmonary veins which initiate reflex cardiac acceleration (Bainbridge's reflex). *Amer. J. Anat.*, 61:203.
56. Nonidez, J. F. (1941). Studies on the innervation of the heart. *Amer. J. Anat.*, 68:151.
57. Olszewski, J. and Baxter, D. (1954). Cytoarchitecture of the human brain stem. Montreal: J. B. Lippincott Company.
58. Page, I. H. and McCubbin, J. W. (1953). Renal vascular and systemic arterial pressure responses to nervous and chemical stimulation of the kidney. *Amer. J. Physiol.*, 173:411.
59. Paintal, A. S. (1953). A study of the right and left atrial receptors. *J. Physiol.*, 120:596.
60. Paintal, A. S. (1953). The conduction velocities of respiratory and cardiovascular afferent fibres in the vagus nerve. *J. Physiol.*, 121:341.
61. Paintal, A. S. (1954). A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst. *J. Physiol.*, 126:255.
62. Paintal, A. S. (1954). The response of gastric stretch receptors and certain other abdominal and thoracic vagal receptors to some drugs. *J. Physiol.*, 126:271.

63. Paintal, A. S. (1955). A study of ventricular pressure and their role in the Bezold reflex. Q. J. Exper. Physiol., XL:348.
64. Paintal, A. S. (1957). Responses from mucosal mechanoreceptors in the small intestine of the cat. J. Physiol., 139:353.
65. Pearce, J. W., and Henry, J. P. (1955). Changes in cardiac afferent nerve fibre discharges induced by hemorrhage and adrenalin. Amer. J. Physiol., 183:650.
66. Pearce, J. W., Henry, J. P. and Chapman, M. (1956). The behaviour and possible functions of cardiac atrial stretch receptors. Abstr. Proc. XX International Congress Physiol., 711.
67. Pearce, J. W., and Whitteridge, D. (1950). The correlation of pulmonary arterial pressure pulses with the discharge of afferent pulmonary vascular fibres in the vagus nerve. J. Physiol., 112:45P.
68. Penfield, W. (Ed.), (1932). Cytology and cellular pathology of the nervous system. Vol.1. New York: Paul B. Hoeber, Inc.
69. Pitts, R. F. (1946). Organization of the respiratory center. Physiol. Rev., 26:609.
70. Pitts, R. F., Magoun, H.W., and Ranson, S. W. (1939). The origin of respiratory rhythmicity. Amer. J. Physiol., 127:654.
71. Pitts, R. F., Magoun, H.W., and Ranson, S. W. (1939). Interrelations of the respiratory centers in the cat. Amer. J. Physiol., 126:689.
72. Pitts, W. (1952). Investigations on synaptic transmission. Josiah Macy, Jr. Foundation Conferences on Cybernetics. Transactions, 9th Conference, 1952.
73. Ranson, S. W., and Billingsley, P. R. (1916). Vasomotor reactions from stimulation of the floor of the fourth ventricle. Amer. J. Physiol., 41:85.
74. Ranson, S. W., Foley, J. O. and Alpert, C.D., (1933). Observations on the structure of the vagus nerve. Amer. J. Anat., 53:289.
75. Skoglund, C. R. (1955). The functional organization of spinal interneurons. Special University lectures in Physiology, University of London.

76. Skoglund, C. R., Haaparen, L. and Kolmodin, G. M. (1958). Membrane and action potentials of spinal interneurons in the cat. *Acta. Physiol. Scand.*, 43:315.
77. Svaetichin, G. (1958). Component analysis of action potentials from single neurons. *Experimental Cell Research, Suppl.*, 5:234.
78. Talov, I. M. (1940). Sensory and motor roots of the IX nerve and the vagus spinal accessory complex. *Arch. Neurol. & Psych.*, 44:1018.
79. Towbin, E. J. (1955). Thirst and hunger behaviour in normal dogs and the effects of vagotomy and sympathectomy. *Amer. J. Physiol.*, 182:377.
80. Wang, S. C. and Ranson, S. W. (1939). Autonomic responses to electrical stimulation of the lower brain stem. *J. Comp. Neurol.*, 71:437.
81. Whitteridge, D. (1948). Afferent nerve fibres from the heart and lungs in the cervical vagus. *J. Physiol.*, 107:496.
82. Wikler, A. (1957). The Relation of Psychiatry to Pharmacology. pp. 85-209. Baltimore: Williams and Wilkins.
83. Winsbury, G. J. (1956). Machine for the production of microelectrodes. *Rev. Scient. Inst.*, 27:514.
84. Woldring, S. and Dirken, M. N. J. (1951). Site and extension of bulbar respiratory center. *J. Neurophysiol.*, 14:227.
85. Woodbury, J. W. and Patton, H. D. (1952). Electrical activity of single spinal cord elements. Cold Harbor Symposium on Quantitative Biology. Vol. XVII, p. 185.
86. Fox, C. A. and Eichman, J. (1959). A rapid method for locating intracerebral electrode tracks. *Staintech.*, 34:39.
87. Freygang, W. H. and Frank, K. (1959). Extracellular potentials from single spinal motoneurons. *J. Gen. Physiol.*, 42:749.

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